D.O.C.E.N.T

(**D**etermining **O**ptimal **C**ashew nut shell liquid **E**xtractio**N** me**T**hod)
Final report project

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Abbreviation list

AA	Anacardic acid
CNS	Cashew nut shells
CNSL	Cashew nut shell liquid
CoE BBE	Centre of Expertise Biobased Economy
FT-IR	Fourier Transform- Infrared spectroscopy
GC	Gas chromatography
HPLC	High-performance liquid chromatography
RBR	Rotating bed reactor
TGA	Thermogravimetric Analysis

Abstract

The production of cashew nuts ($2E^9$ kg/y) generates an annual waste stream of $3E^9$ kg, in the form of cashew nut shells (CNS). These CNS contain a valuable liquid component: cashew nut shell liquid (CNSL). This brown viscous liquid contains, amongst others, anacardic acids and cardonol, which find use in the medical and coating industry, respectively. The goal of this project was to determine the optimal extraction conditions of cashew nut shell liquid (CNSL) out of cashew nut shells (CNS), while maintaining an economically attractive and technically feasible process. The desired yield for the extraction was ≥90 m%. The project started with three extraction techniques in mind; a rotating bed reactor, accelerated solvent extraction and supercritical fluid extraction. Since the bed reactor showed promising results in early stages, a yield of over 70 m%, in relation to the total amount of CNSL present, this was chosen as the extraction method to optimize.

The parameters used for the project were extraction temperature, particle size distribution, stirring speed, extraction time, rotating bed or propeller stirrer, solvent and solid/solvent ratio. The resulting extract was examined using HPLC, TGA, FTIR and GC. The parameters that were found to influence the extraction yield: extraction time, solvent use, solid/solvent ratio and extraction temperature. It was found that the optimal extraction time, in relation to the yield gained afterwards, is one hour with a yield of 0,40 g CNSL/g CNS. After two hours of extraction, there was an increase of yield below one percent, which determined the optimum. It was also found, that removing the rotating bed, and using a mixer in the reactor, did not result in deviations in yield. The influence of temperature was visible, with 50 ºC and 70 ºC showing similar results, with a yield of 0,40 g CNSL/g CNS. This was while 20 ºC showed a result of 0,33 g CNSL/g CNS, therefore 50 ºC was found to be optimal. When comparing the solid/solvent ratios 1:7, 1:13 and 1:20, it was found to give a yield of 0.28, 0.33 and 0.39 g CNSL/g CNS, respectively. Finally, the use of the solvents heptane and ethanol were examined, which showed the following: Heptane showed a yield of 0,36 g CNSL/g CNS, while ethanol yielded 0,40 g CNSL/g CNS under similar conditions. In summary, the optimal extraction conditions, when taking influentory parameters in mind, is using ethanol at 50 ºC, with 1:20 solid/solvent ratio, for one hour.

TGA analysis showed that, when comparing ‘fresh’ CNS and CNS that underwent extraction, an extraction rate of 78.2 m% was achieved, therefore not obtaining the 90 m% goal of the project. In order to assess the ethanol fraction left in the CNSL after vacuum distillation, GC-analysis was executed, which showed an average of 10 % (v/v) remaining in the CNSL. The yield was not adjusted for ethanol content, since there was no data on the amount of solvent in the extractions, where heptane was used. Further analysis, in order to determine the composition of the CNSL, in the form of HPLC and FTIR, was also performed.

Literature shows [2] an AA content in CNSL differing between 50-70 m%, when extracted under mild conditions. HPLC-analysis showed the absence of saturated AA, while AA triene was present in concentrations around 83 mg/ml. However, due to the lack of mono- and diene AA analytical standards, the content of these substances in the CNSL could not be determined. AA’s were extracted out of CNSL, by dissolving it in 5% aqueous methanol and excess calcium hydroxide, which formed the precipitate calcium anacardate. This was then further purified, by suspending it in hydrochloric acid (1.5M) and washing it with ethyl acetate and distilled water. The solids were then dried and analyzed by FTIR and HPLC. The data of the extract corresponded with that of magnesium sulphate, which was used as a drying agent during the extraction, therefore, its effectiveness was called into question.

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Contents

1. Introduction

With the combat against global warming and pollution, research towards bio-based sources of valuable components has increased over the last decades [1]. Bio-based sources come in many forms, like sugar-based feedstock such as sucrose and starch, but also microbial feedstock, like bacteria and fungi. This way, the dependence of the finite fossil resources can be decreased and the distorting effects towards the climate can be decreased.

This project is done on behalf of, and in collaboration with the research group Center of Expertise, Bio-Based Economy, (CoE BBE), which has previously put effort towards the research around Cashew nut shell liquid (CNSL). Previous work involved extraction by pyrolysis, while this project lays the focus on solvent extractions. The composition of the CNSL is mostly phenolic acids (~80 m%) [2] but is dependent on the extraction route [3]. The relevant liquid composition for this report is CNSL extracted by (natural) solvent, which contains mostly anacardic acids (saturated, mono-, di- or triene) and cardol [3]. AA triene is known for its anti-inflammatory and anti-oxidizing properties and is most prominently (~44 m% of AA content) available in CSNL [4], while the versatile liquid as a whole can also be used as fuel source [5], additive [6] or paint. Even though this research mainly focused on extraction, the market potential for CNSL and its compounds are promising.

The main goal of this project was to design an extraction process, which could extract CNSL from cashew nut shells (CNS) with a yield of ≥90 m%. The prerequisite was that the process was technically feasible and economically attractive. In order to achieve this, mild conditions were chosen, such as temperatures between 20 and 70 °C and (retractable) solvents, like ethanol and heptane. Several extraction methods were selected: a reactor with a rotating bed (RBR) or agitator (propeller stirrer), accelerated solvent extraction (ASE) and supercritical CO_2 extraction (SC-CO_2). Due to the effectiveness of the RBR, the other methods were not executed.

This report consists of seven chapters, first the background information and the motivation for this project will be construed. Then in the theoretical background, additional information, requisite for the progression of the report is given. Afterwards, the used materials and methods will be illustrated. Subsequently, all results with accompanying visual guidance will be interpreted, after which these results will be discussed in the discussion. Following, the conclusions will be presented, and corresponding recommendations will be outlined afterwards.

2. Theoretical background

2.1. Cashew nut shell liquid (CNSL)

A cashew nut comes from a tree that produces a cashew seed and a cashew apple. A shell surrounds the cashew nut, or kernel, (see figure 1). Only the cashew nut itself is used for consumption. The shell of the cashew nut seed is, amongst others, used for paints and lubricants. The nuts are sold without the shell because the shell contains compounds that can cause an allergic reaction or an irritation. The cashew nut shell, CNS, is leathery and contains a brown viscous liquid, CNSL or the pericarp fluid, which amounts for ~67 m% of the nut weight. Depending on the method of obtainment, CNSL may present a different chemical composition and can therefore be classified into two main types: solvent-extracted CNSL and technical CNSL. The CNSL contains, among others, phenolic lipids, anacardic acids, and cardanol. This project focuses on the natural CNSL, the solvent extracted CNSL. [7]

Natural CNSL is obtained by using some solvent extraction techniques (like Soxhlet, supercritical carbon dioxide, or subcritical water) in order to obtain the components without inducing any chemical modifications. With these techniques, natural CNSL represents the original composition found in nature, which is composed by AAs (60-70%), cardols (10-20 m%), cardanols (3-10 m%), 2-methylcardols (2-5 m%), and other minor components.[8]

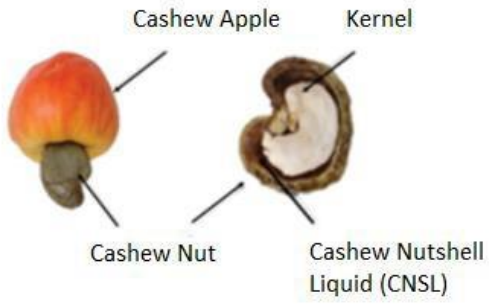

Figure 1: Profile of a cashew nut. [7]

2.2. Extraction set-ups

2.1.1. Rotating bed reactor (RBR)

A rotating bed reactor holds a rotating packed bed with a solid phase (CNS) as displayed in figure 2. The solid phase remains in the bed by a filter. By rotating the bed at high speed, the extraction solvent will be aspirated from the bottom and top of the vessel to the middle and through centrifugal force pushed outward through the solid phase. This maximizes axial mixing and convective transport. The resulting efficient mass transfer minimizes extraction time and enables a high yield. [9]

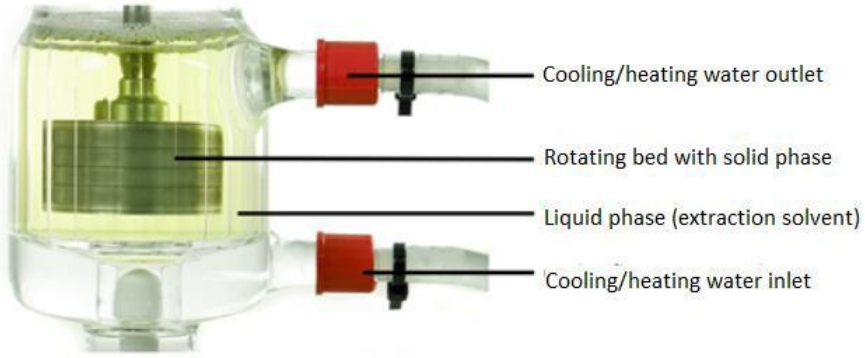

Figure 2: Rotating bed reactor with a cooling system for batch process. [9]

2.1.2. Agitator

For checking the advantages of the rotating bed compared to a regular propeller stirrer (hereafter: 'regular stirrer') for agitation, extractions will also be executed using a propeller stirrer (figure 3). The CNS will be on the bottom of the reactor, therefore the solids need to be separated from the liquid after the extraction by filtration.

Figure 3: Regular propeller stirrer

2.3. General concept of solid-liquid extraction

In solid-liquid extraction, molecules from a solid particle are extracted by the liquid phase. The speed of reaching the extraction equilibrium depends on many variables, such as temperature, particle size, solvent, solid/solvent ratio and the concentration difference. Transport of molecules to the liquid is dependent on the affinity of the molecules to the solvent. With a higher temperature, the mass transfer increases and is more efficiently due to a higher solubility of CNSL in the solvent. If the rotation speed increases, convection increases, resulting in faster mass transfer through the external film layer [10]. When particles are smaller, there is more contact area for the solvent, which should also result in a higher yield. When there is more liquid than solid, the extraction should also proceed faster, because the concentration of the extract is lower, thus creating a bigger concentration difference [11].

2.4. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis, TGA (figure 3), is an analytical technique that can measure the loss of mass of a sample over time, while the temperature is increased. When using this technique, the temperature is increased, ranging from 20 to 1000°C. The boiling point of CNSL (215 °C) is lower than the other components in the CNSL, like hemicellulose (277 ºC), cellulose (327 ºC), lignin (387 ºC) and polymeric material (430 ºC). At the specific boiling points of the components, the components start to evaporate. As a result, the mass changes, which is measured by a precision balance.

By using TGA, the amount of CNSL in the shell can be determined. Therefore, the extraction yield can be determined.[12]

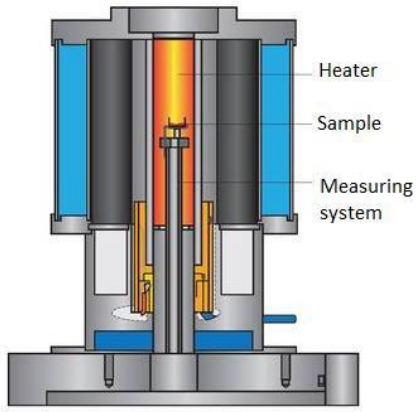

Figure 4: Schematic drawing of the inside of a TGA.[12]

2.5. Anacardic acid

AA is a compound that can be used for medicinal purposes. In a pure form it is an expensive ($1000/10 mg for AA triene) product. AA can be present in 4 different forms: saturated, with one (mono-), two (di-) and three (tri) double bonds (ene). The mono-, di- and tri- ene are the ones present in the CNSL. AA's structure formula looks similar to cardol, 2-methylcardol and cardanol, three molecules that are also present in CNSL (figure 5).

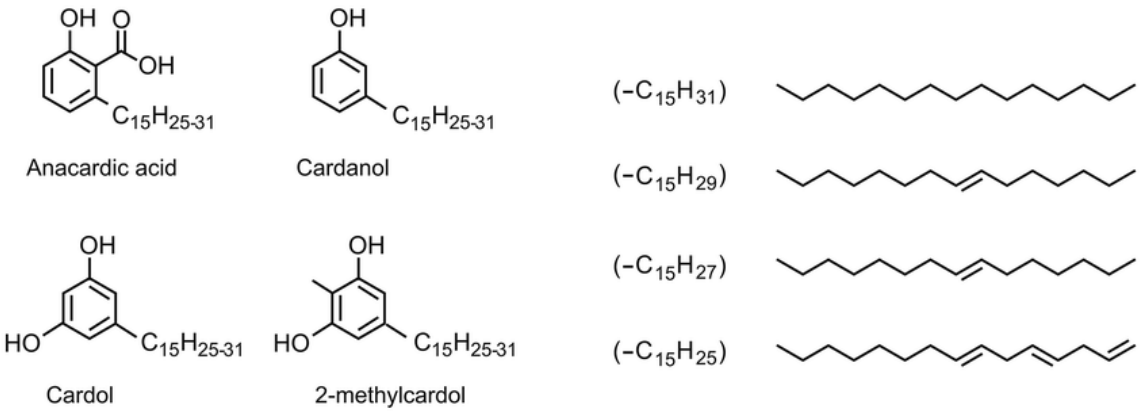

Figure 5: AA and its similar forms in CNSL: cardanol, cardol and 2-methylcardol.

The AA is recognized by its carboxylic acid group (COOH) attached to the benzene ring. This acid group can be easily detected using FT-IR analysis. Using HPLC analysis, the different compounds can be detected due to their different polarities, i.e. different affinity with the column. This is also the case for the three forms of AA present in the CNSL. These will be detected separately due to the mono-, di- and tri-ene bonds resulting in different polarities.

3. Methods

3.1. CNS preparation

CNS obtained from CoE BBE, was pre-treated using liquid nitrogen and grinded using a mortar. Particles of 2-4 mm were collected using metal sieves and brought to room temperature before use.

3.2. Extraction of CNSL

Numerous extractions were performed in this project following a similar process, which is described in figure 6. First, a solid/liquid extraction was performed after which the liquid phase got concentrated by evaporation and the solid phase was dried in open air. Figure 7 shows the general process of the performed CNSL extractions with a regular stirrer, in this process there was an extra filtration step.

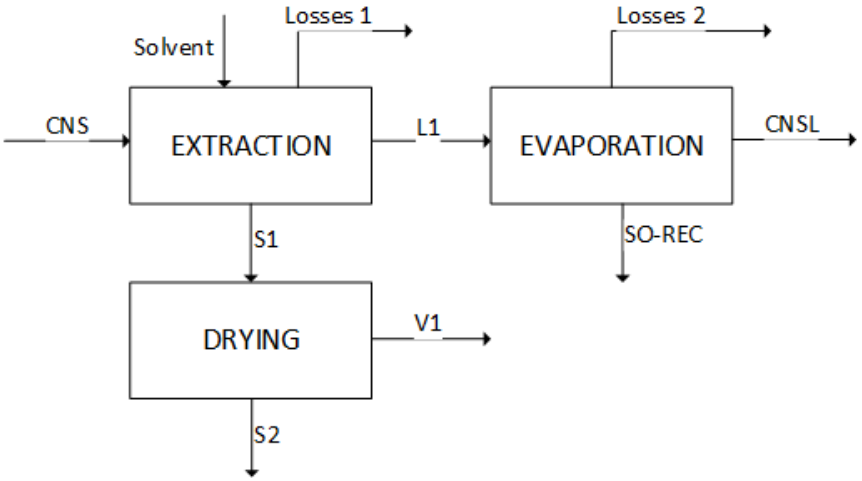

Figure 6: General process of performed CNSL extractions with the RBR, with accompanied stream names

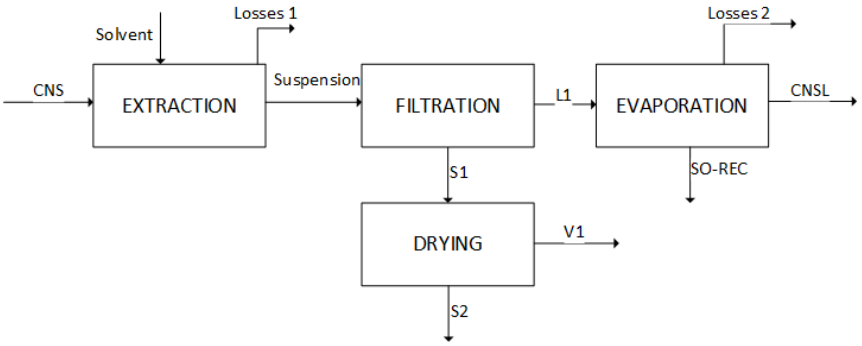

Figure 7: General process of performed CNSL extractions with the regular stirrer, with accompanied stream names

RBR extractions were performed using a Spinchem rotating bed, placed in a double-walled reactor heated by a water bath with overhead stirrer. For every RBR extraction, 7,5 g of CNS was used and divided equally over the bed. The rotating bed was weighed completely before placement in the reactor. The water bath was heated to the desired temperature. Next, the rotating bed was placed in the reactor and set to the desired rotating speed. Finally, the pre-weighed solvent was added. Extraction time was tracked using a stopwatch. After extraction the liquid (L1) was collected in a round bottom flasks and

weighed. The rotating bed was weighed again in order to determine the mass of the solid fraction after extraction (S1).

For extractions using a regular stirrer, the same set-up and Spinchem reactor was used. Only the rotating bed was replaced with a regular stirrer. And the 7,5 g CNS was placed loosely in the reactor. After an extraction, the total reactor content was filtered using a pre-weighed 12 µm filter. When filtration was finished, the filter was weighed again in order to determine the solid weight (S1). The liquid was collected in a round bottom flask and weighed (L1).

All the resulting liquid fractions (L1) were concentrated under reduced pressure (118 mbar) using a rotating evaporator at 45°C, for approximately 40 minutes. The recovered solvent (SO-REC) was weighed, as well as the remaining CNSL mass. The CNSL was stored in a refrigerator at 7°C. The resulting solid fractions (S1) were dried in a fume hood (in open air) for at least 24 hours. After that the CNS residue (S2) was re-weighed and stored at room temperature.

The “standard extraction” is defined by extracting for 60 minutes at 50 °C using 7,5 g CNS (2-4 mm) and 150 ml of ethanol as solvent. The bed and stirrer were rotating at 300 rpm. Single adjustments were made on this ‘standard’, in order to compare the influence of the parameter in question. Table 1 shows the alternations in parameters and all the performed extractions. Every extraction with unique parameters was performed in twofold.

A TGA was executed on the CNS residue (S2) from a RBR-extraction of 1 hour and one of 10 minutes. Furthermore, TGA was performed for a 1 hour extraction with a regular stirrer. For this analysis, the CNS residue (S2) of the corresponding samples was vacuum dried beforehand. The resulting CNSL from an 1 hour RBR-extraction with ethanol and an extraction with Heptane were analysed using FTIR.

Table 1: different parameters that were investigated for the extraction of CNSL from CNS

Parameter	**Standard condition**	**Other conditions tested**
Particle size	2-4 mm	6-10 mm
Solvent	Ethanol	Heptane
Stirring speed	300 rpm	0 and 1150 rpm
Extraction set-up	Rotating bed	regular stirrer
solvent/solid ratio and set-up	20 ml/g and regular stirrer	6,67 and 13,33 ml/g with regular stirrer
Temperature	50 °C	20 and 70 °C
Time	60 minutes	10, 20, 30, 40, 50 and 120 minutes
Stirrer and time	Regular stirrer	2, 4, 8, 10, 20, 30, 40, 50 and 60 minutes

3.3. CNSL composition

3.3.1 Anacardic acid content by HPLC

To determine the AA content of the derived CNSL, HPLC-analysis was performed. Using a reverse phase C18-column at 25 °C. The mobile phase contained 80 %(v/v) acetonitrile and 20 %(v/v) Milli Q with 3 %(v/v) acetic acid at pH 3,0 with a flow of 1,8 ml/min. The injected sample volume was 20 µl. The wavelength of the chromatograms was measured at 280 nm and the UV spectra from 200-400 nm. All CNSL samples were diluted 1000 times in ethanol and filtered using an 0,45 µm filter, before injection. For the calibration curve, an AA solution in ethanol was injected with the following concentrations: 0, 50,100, 200 and 500 µg/ml.

3.3.2. Ethanol content of CNSL

The ethanol content in the CNSL was determined using GC-analysis. 1.5 ml sample was placed in a 20 ml glass flask with crimp top and heated in an oven for 10 minutes at 50 ºC. After heating, 1 ml of vapour was retracted from the headspace and injected in the GC to determine the ethanol content. In order to determine the ethanol content, a calibration curve was drafted with 0, 5, 10, 20 and 50 %(v/v) of ethanol/water.

3.3.3. Isolation of anacardic acid

5 grams of CNSL was dissolved in 30 ml 5% aqueous methanol, under stirring while 2,5 grams of calcium hydroxide was added in portions. The mixture was continuously stirred for 3 hours at a temperature of 50 °C, where the AA reacts with the calcium, forming calcium anacardate, as explained in the following chemical equation:

$$Ca(OH)_2 + 2\,AA \rightarrow\ Ca^{2+} + 2OH^- + 2\,AA \rightarrow Ca(AA)_2 + 2\,H_2O$$

With a filter, the precipitated calcium anacardate was removed and washed with methanol. After the washing, the cake was dried in a vacuum oven (approx.. 10 mbar) at 50 °C for 2 hours. The calcium anacardate was resuspended in 3 ml 11M HCl and 22 ml distilled water and stirred for 1 hour at room temperature. The mixture was washed in a separatory funnel twice with 10 ml ethyl acetate, afterwards the organic layer was washed twice with 5ml demineralized water. The washed organic layer was dried over anhydrous magnesium sulphate for 30 min and concentrated under reduced pressure (150 mbar). The extracted AA and the CNSL were analysed using FT-IR and HPLC [13].

3.3.4. FT-IR analysis

FT-IR analysis was performed in order to determine the presence of AA in the samples. As previously explained in chapter 2.5, the carboxylic acid groups present in the three different forms of AA can be determined. FT-IR shows peeks at wavenumbers 2800 and 2900 (cm^{-1}) when carboxylic acid groups are present.

4. Results

4.1. Extraction performance

The ‘standard extraction’ is defined as following: using the rotating bed, 300 rpm stirring speed, 50 ºC, 7,5 g CNS, with a particle size of 2-4 mm using 150 ml ethanol. This extraction showed an average CNSL recovery of 0,39 grams per gram of CNS used. The standard deviation of the two measurements is only 0,012. In figure 8, the influence of using heptane as a solvent is shown, with a standard deviation of 0,019. The use of heptane results in a lower recovery of 0,36 g CNSL/ g CNS.

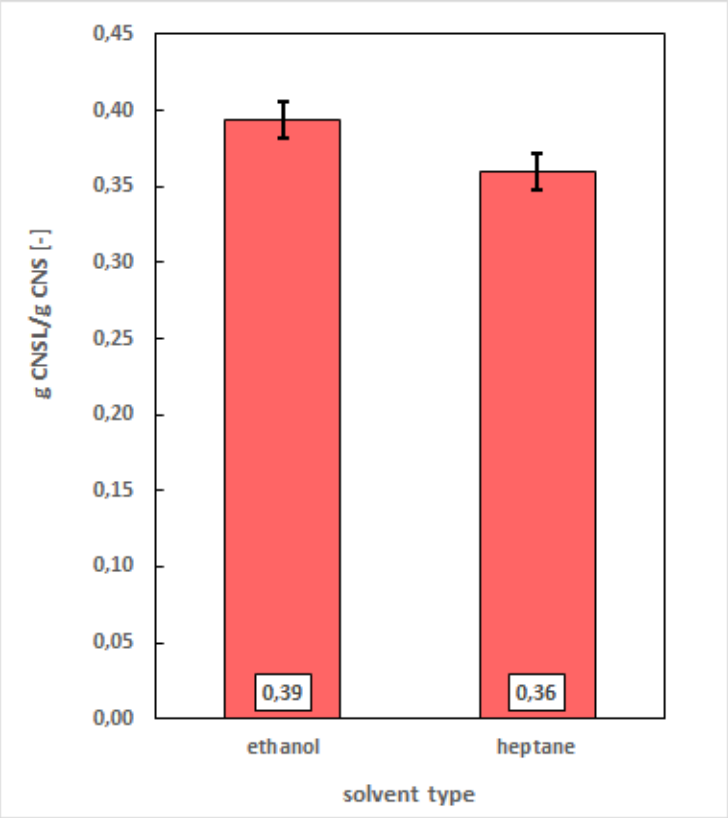

Figure 8: g CNSL/ g CNS found for extraction performed with different solvents (for a 1-hour extraction at 50°C in RBR with 7,5 g CNS, particle size 2-4 mm, rotating speed 300 rpm, and 150 ml solvent.)

In figure 9 the extraction performance using a different particle size distribution is shown. 2-4 or 6-10 mm particles were used, which showed a standard deviation of 0,012 and 0,010, respectively. The particle size does not have a significant influence on CNSL recovery under these conditions.

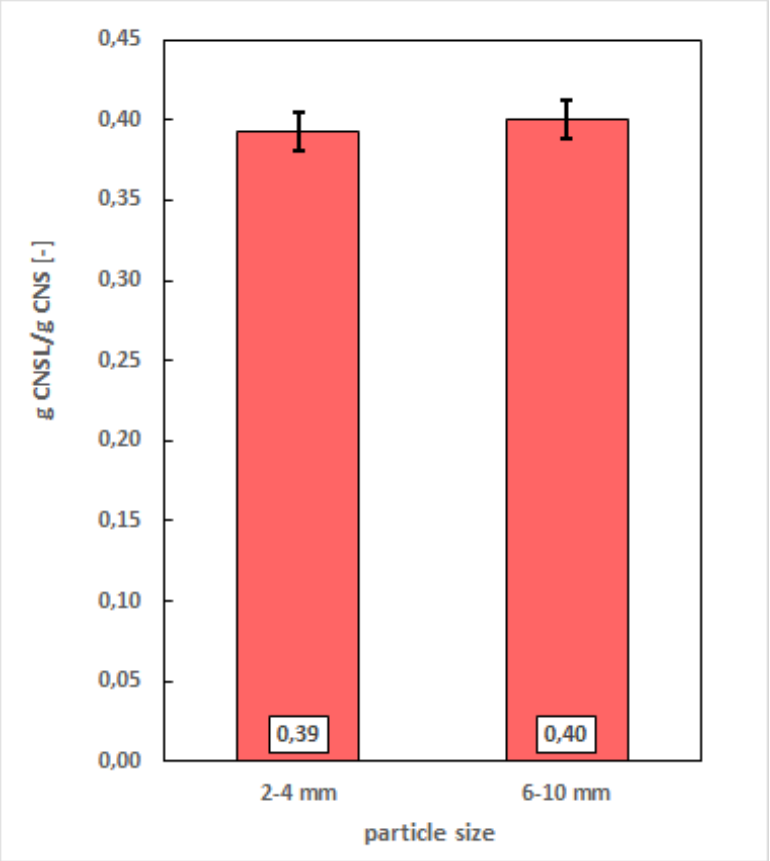

Figure 9: g CNSL/ g CNS found for extraction performed with particle sizes (for a 1-hour extraction at 50°C in RBR with 7,5 g CNS, rotating speed 300 rpm, and 150 ml ethanol as solvent.)

Figure 10 shows the recovery of g CNSL/ g CNS that has been set out against the solvent/solid ratios of 6,67, 13,33 and 20 ml/g. The values g CNSL/ g CNS yield are 0,28, 0,33 and 0,39 with standard deviations of 0,019, 0,090 and 0,012, respectively. The use of a higher solvent/solid ratio gives a higher yield of g CNSL / g CNS. The correlation between these two variables for a solvent/solid ratio between 6,67 and 20 ml/g seems to be linear and can be expressed as equation X. Equation 1 has a R^2 of 0,99.

Equation 1: $$g\ CNSL/g\ CNS = 0{,}0085*(\text{Solvent/solid ratio}) + 0{,}22$$

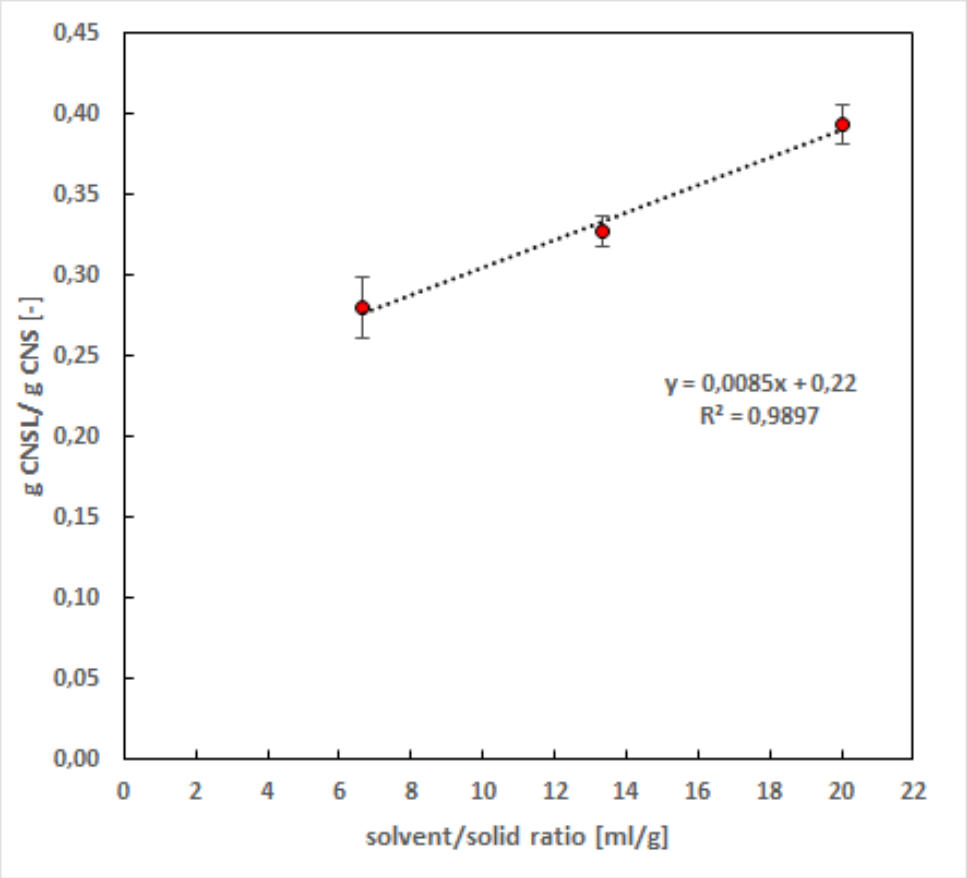

Figure 10: g CNSL/g CNS found for extraction performed with different solid/solvent ratios (for a 1-hour extraction at 50°C regular stirrer with 7,5 g CNS, particle size 2-4 mm, rotating speed 300 rpm and ethanol as solvent.)

Figure 11 shows the influence of rotating on the yield of CNSL per g CNS.
For rotating speeds of 0, 300 and 1050 rpm a yield of 0,35, 0,39 and 0,36 were found with standard deviations of 0,010, 0,009 and 0,018, respectively. There is no clear correlation between the rotating speed and the CNSL yield.

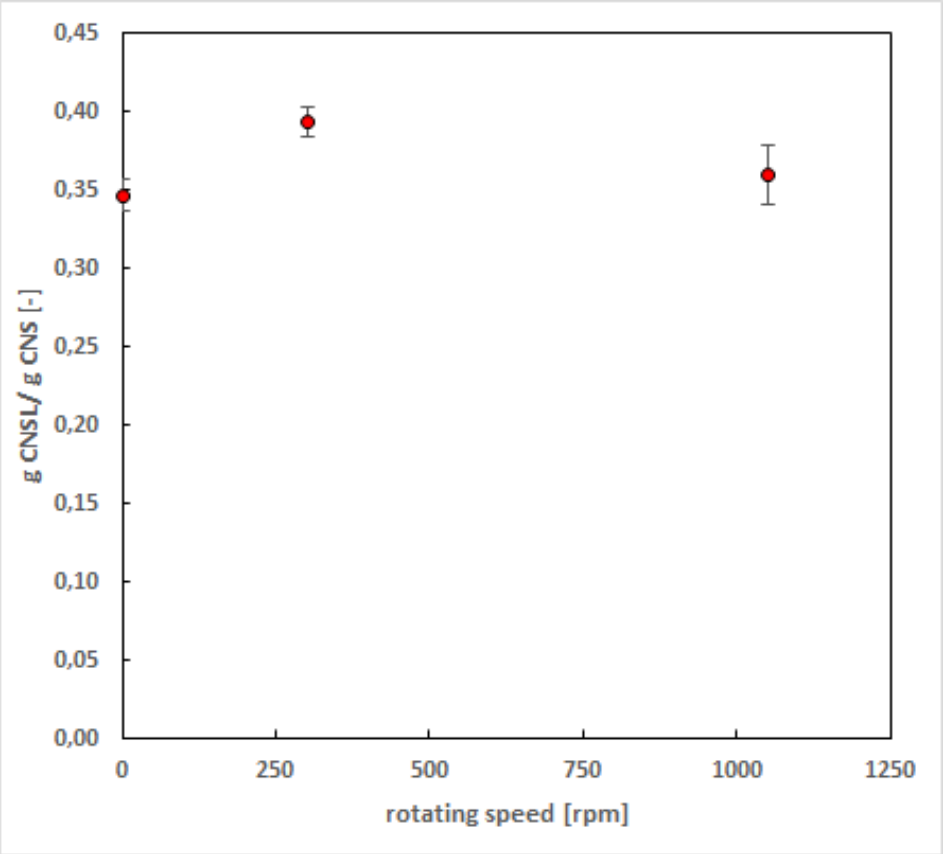

Figure 11: g CNSL/g CNS found for extraction performed with different rotation speeds (for a 1-hour extraction at 50°C in RBR with 7,5 g CNS, particle size 2-4 mm and 150 ml ethanol as solvent.

Figure 12 shows the influence of temperature on the yield of g CNSL per g CNS. For temperatures of 20, 50 and 70 ºC a yield of 0,33, 0,39 and 0,40 were found with standard deviations of 0,009, 0,012 and 0,037, respectively. This shows that lowering the temperature has a negative effect on the CNSL yield per gram CNS. However, increasing the temperature from 50 to 70 ºC did not give a clear raise in CNSL yield per g CNS.

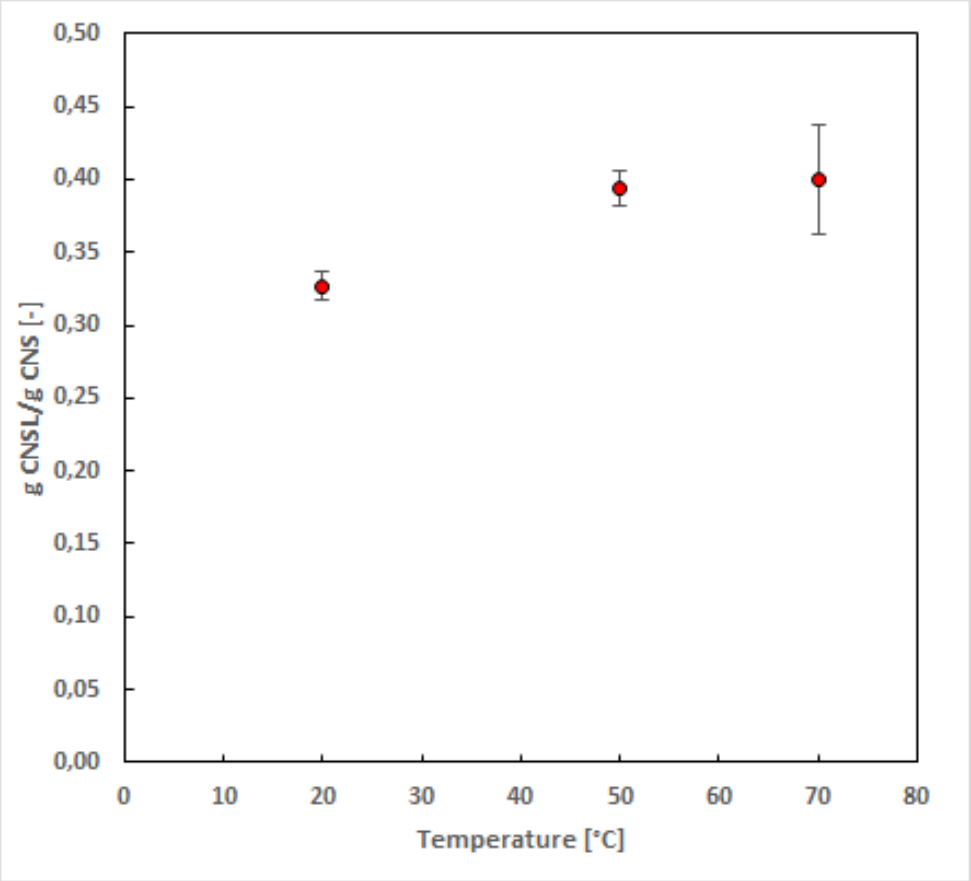

Figure 12: g CNSL/g CNS found for extractions performed at different temperature (for a 1-hour extraction at 50°C in RBR with 7,5 g CNS, particle size 2-4 mm, rotating speed 300 rpm and 150 ml ethanol as solvent.)

The rotating bed showed different extraction kinetics when compared to using a regular stirrer as displayed in figure 13. In this figure, the yield of g CNSL per g of CNS is set out against time. The kinetics for the rotating bed is expressed in equation 2, with *t* as time in minutes.

Equation 2: $$g\ CNSL/g\ CNS = 0{,}20t^{0{,}16} \quad (R^2 = 0{,}96)$$

The kinetics of the extraction with a regular stirrer is expressed in equation 3, with *t* as time in minutes. In early stages, the yield of g CNSL per g CNS is higher than using a rotating bed reactor, but for extractions times after 45 minutes the RBR performs better than the regular stirrer does.

Equation 3: $$g\ CNSL/g\ CNS = 0{,}27t^{0{,}076} \quad (R^2 = 0{,}89)$$

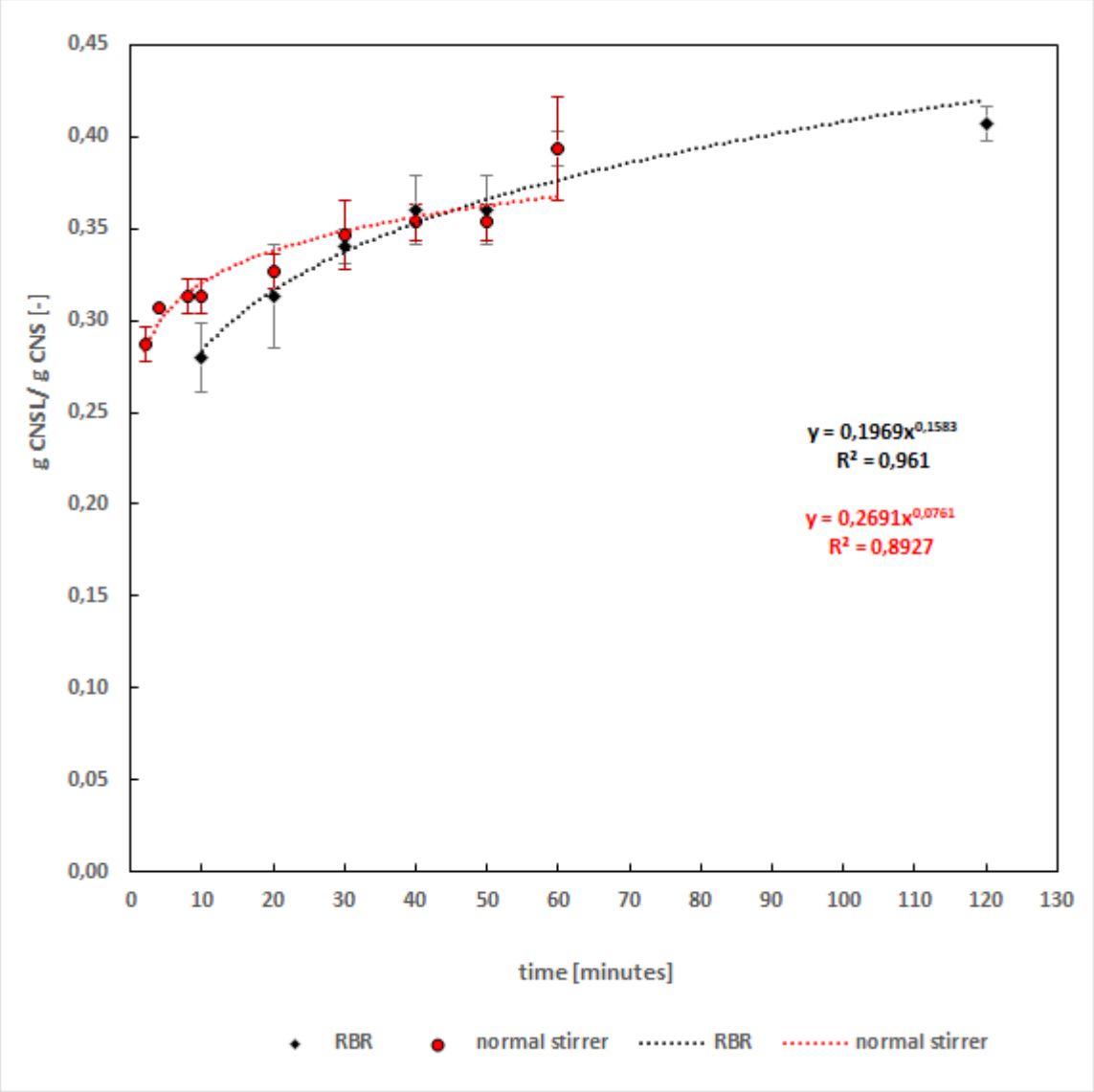

Figure 13: g CNSL/g CNS found at different extraction times (for a extraction at 50°C in RBR with 7,5 g CNS, particle size 2-4 mm, rotating speed 300 rpm and 150 ml ethanol as solvent.)

4.2. Mass balance

The mass balance was drafted over three steps: extraction, evaporation and drying. The tables with the total difference mass balances can be found in appendix 1.

With the extraction mass balance, the losses in the reactor (Losses 1) can be determined. These losses are mainly ethanol that evaporated during the extraction. The value of the 'Losses 1' is calculated as following: 'Losses 1' = (CNS + solvent) - (L1 + S1). The lowest value of Losses 1 (2,85 g) is with a particle size of 6-10 mm. The highest value of the 'Losses 1' (17,2 g) is with the regular stirrer. The losses of the regular stirrer are higher, due to filtration, where (pure) ethanol easily evaporates.

With the evaporation mass balance, the losses during the evaporation (Losses 2) can be determined. The value of 'Losses 2' is calculated as: 'Losses 2' = (L1) - (CNSL + SO-REC). Some samples have a negative loss during evaporation, which is expected to be caused by an improper emptied recovery flask or due to measurement errors.

The drying mass balance was performed for the experiments that were supported by TGA-analysis. In this balance, the vapour that is going to be solvent, is calculated as V1 = S2 – S1.

In summary, the total amount of losses is described as 'Total losses' = Losses 1 + Losses 2 + V1.
This calculation is only possible for the experiments supported by TGA-analysis, which are shown in table 2.

Table 2: Total losses during the extraction process

parameter	condition	Duplo	L1 (g)	L2 (g)	V1 (g)	L TOTAL (g)
none	standard	1	4,6	3,4	5,3	13,3
none	standard	2	5,1	0,1	5,2	10,4
set-up	regular stirrer	1	16,9	4,9	2,9	24,7
set-up	regular stirrer	2	17,5	25,1	2,5	45,1
time	10 min	1	2,6	2,4	3,3	8,3
time	10 min	2	2,7	1,9	3,9	8,5

Table 2 shows that the highest loss are with the regular stirrer setup, with an average of 34,9 g. Per expectation, decreasing extraction time results in the lowest loss with an average of 8,4 g. The average recovery of ethanol, after solvent removal in all experiments performed with 150 ml ethanol and 7,5 g CNS, averaged at 89,4 m% with a 95% confidence interval between 9,8-11,4 m%.

4.3. Compound analyses

4.3.1. High-performance liquid chromatography (HPLC)

HPLC analysis was executed on the following samples: CNSL gained by solvent extraction, AA triene , technical CNSL and cardanol.

The chromatogram shows prominent peaks in the first minute. Using uracil as a known standard, the peaks in the first minute were recognized as so called “injection peaks”. This injection peak could be caused by the solvent or the mobile phase [14]. Therefore, in all chromatograms, the first peak was ignored.

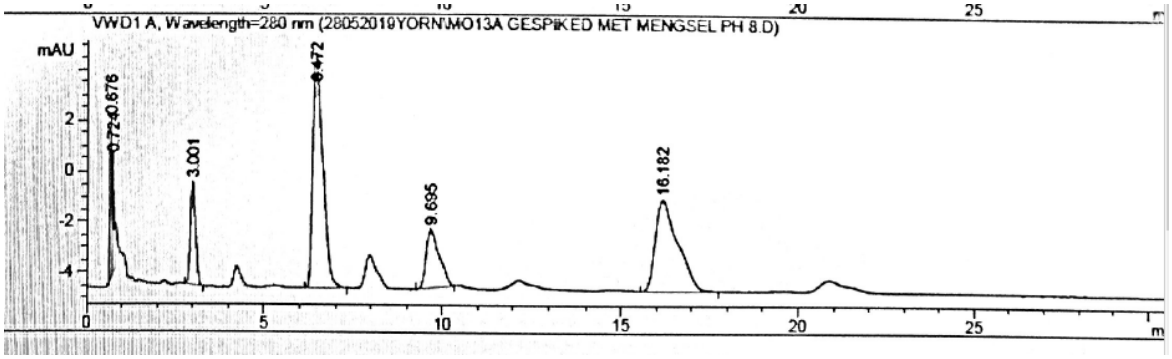

Figure 14: Sample 30 minutes, 7,5 grams CNS, 150 ml ethanol and 50 °C in RBR

Figure 14 shows clear differentiation between distinct peaks, which allows for easy distinction of the compounds present, further in this chapter .

Appendix 2 shows the graphs, retention times and areas of the AA triene peaks of 0,05, 0,1, 0,2, 0,5 mg/ml. There is a prominent peak at 6,48 min in the CNSL chromatogram, that matches with the peak of the sample at 6,47 min. Therefore, it was concluded that AA triene is present in the CNSL.

As displayed per figure 15, a calibration line of AA triene was drafted with the concentrations 0, 0.05, 0.1, 0.2 and 0.5 g/L.

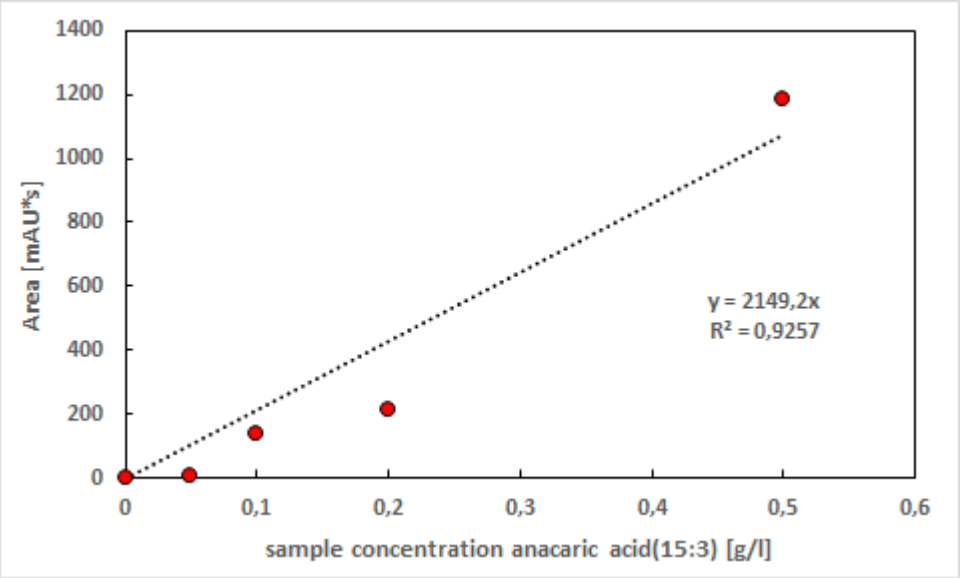

Figure 15: Calibration line of AA triene

The trend line of the calibration line makes it possible to detect the relation between the area of the peak and the concentration of the sample.

Equation 4: $$\boldsymbol{Peak\ area = 2149,2 * concentration\ Anacardic\ acid \quad R^2 = 0,93}$$

For the sample nº 16, the concentration of AA triene is 0,083 mg/ml. However, it must considered that the sample is diluted 1000x. Therefore, the real concentration of AA triene is 83,02 mg/ml.

To identify other compounds, additional HPLC-analyses were executed. Figure 16 shows the chromatogram of technical CNSL.

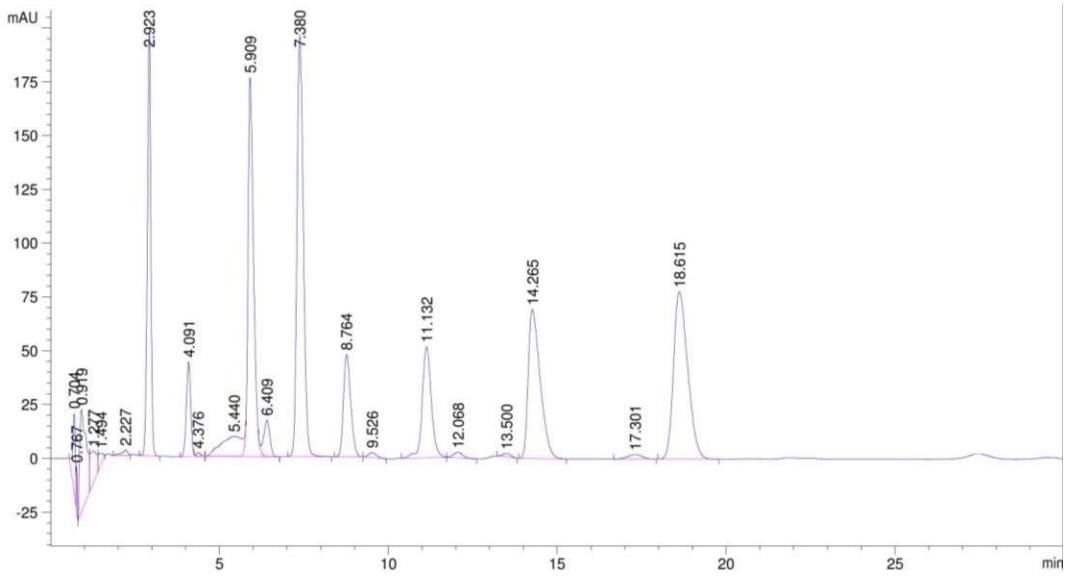

Figure 16: HPLC-chromatogram of technical CNSL.

The main compounds of technical CNSL are cardanol and cardol. In figure 17, the chromatogram of cardanol is shown to exclude other larger peaks in the chromatograms. The higher peaks are at 7.3, 11 and 18 min, these are presumed to be the cardanol tri, di and monoene, respectively.

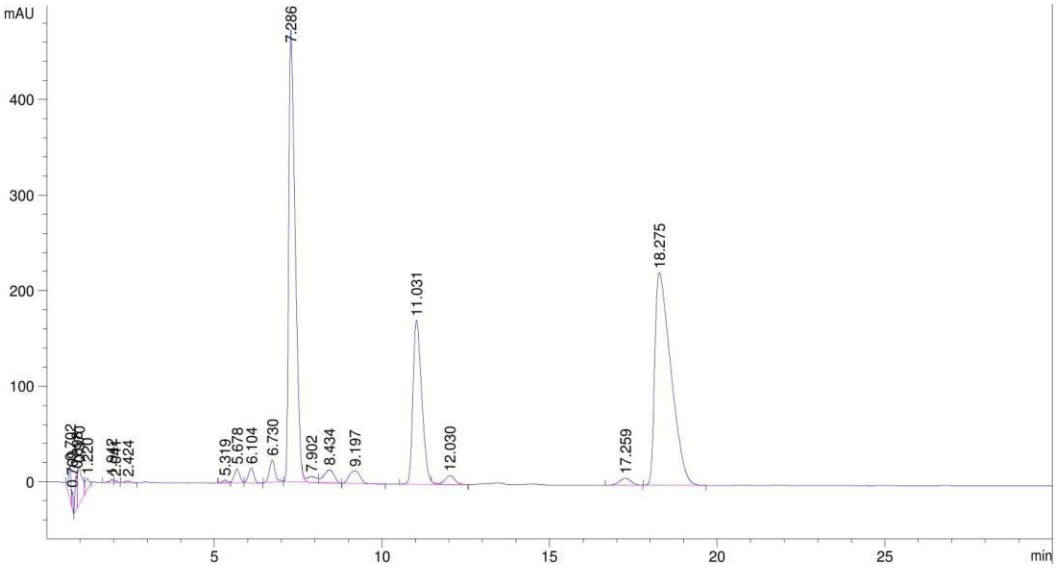

Figure 17: HPLC chromatogram of cardanol.

Figure 18 shows that the retention time of saturated AA is at 23,5 min.

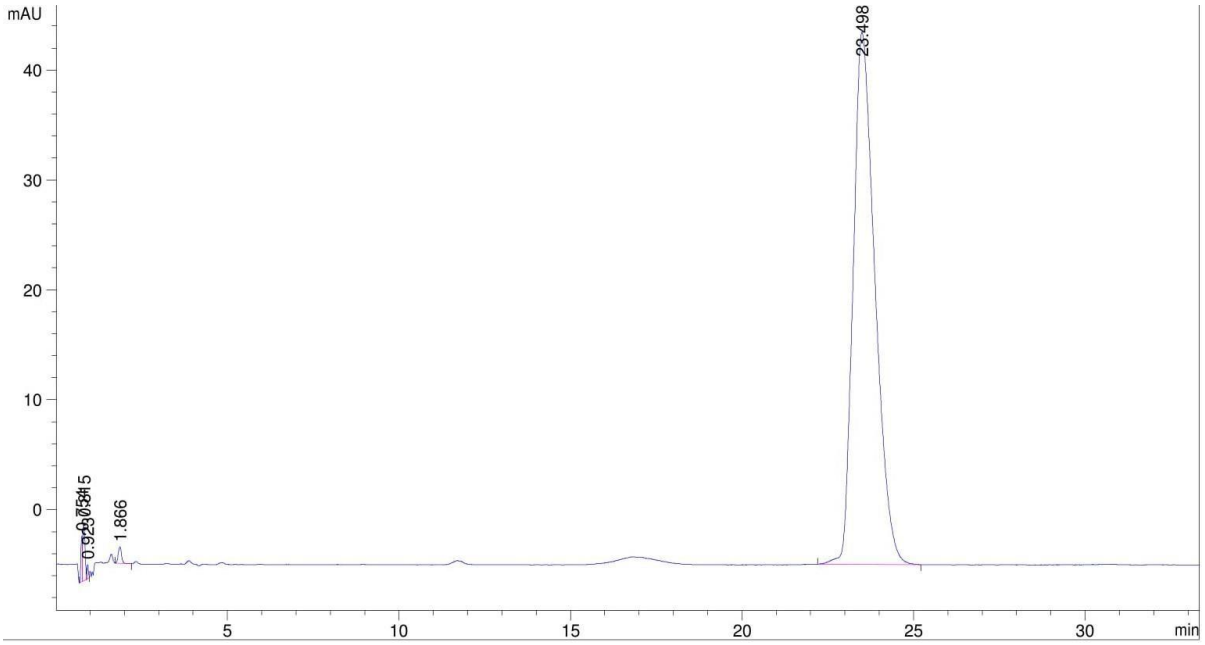

Figure 18: Saturated AA analytical standard

In figure 19, the HPLC chromatogram of the AAs, isolated from the CNSL gained by solvent extraction is shown. No high peaks are visible in the chromatogram. The peaks that are visible (retention time 6,026; 7,458; 11,857), are also evident in the chromatogram of the technical CNSL.

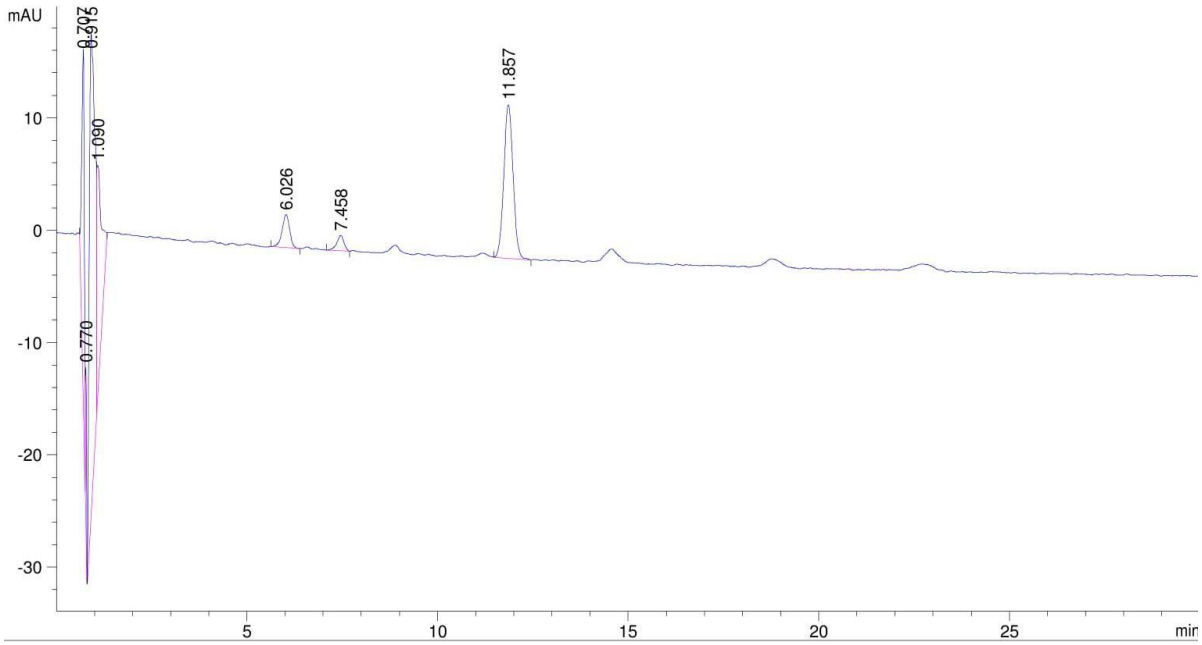

Figure 19: HPLC chromatogram of the AA, isolated from the CNSL gained by solvent extraction.

No AA can be present in technical CNSL, since due to the high pyrolysis temperatures, AA decarboxylates to cardanol. When comparing this chromatogram with the chromatogram of technical CNSL, all peaks match, therefore it can be concluded that no AA is present in the sample. With this data, all peaks in figure 20 (14) can be identified.

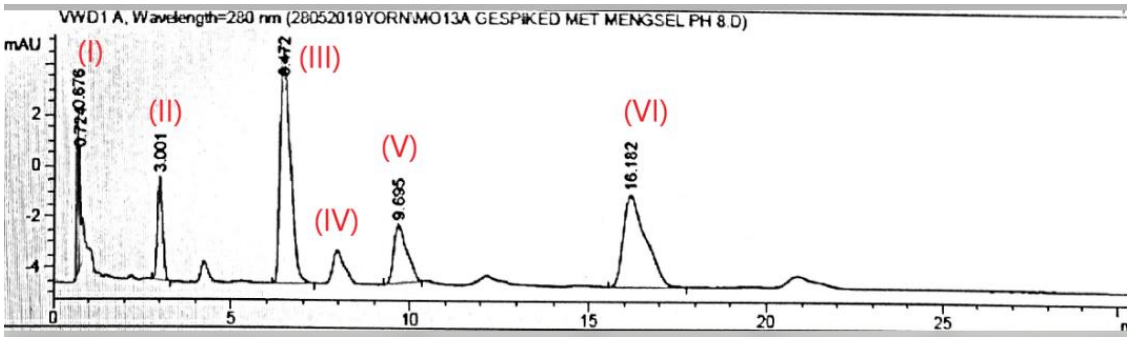

Figure 20: Identification of the peaks:(I) injection peak; (II) Cardol at 3 min; (III) AA triene at 6,5 min; (IV) Cardanol at 7,3 min; (V) AA diene at 9,7 min and (VI) AA monoene at 16,2 min.

Now, according to literature [15] and HPLC-analysis of the AA triene standard, cardanol and the technical CSNL, it is possible to identify the peaks. The (I) is the injection peak, while (II) is cardanol because when comparing to the retention times of the technical CNSL, there is a peak that matches at 3 min. The (III) is AA triene according to the standard. The (IV) is cardanol according due to equal retention times when analysing pure cardanol. The other two peaks are AA diene (V) and AA monoene (VI), the AA diene leaves the column before the monoene, due to its polarity. Additionally, HPLC-analysis of saturated AA standard was performed, showing a retention time of 23 min, therefore it can be concluded that no saturated AA is present in the CNSL.

4.3.2. Fourier-transform infrared spectroscopy (FT-IR)

In figure 20, strong peaks at wavenumber 2900 and 2800 (cm^{-1}) indicate the presence of carboxylic acid groups, which AAs contains. The FT-IR analysis also indicates there is an acid present in the CNSL. The peak at 1700 (cm^{-1}), indicates C=O bonds which match the double bonds in the diene AA.

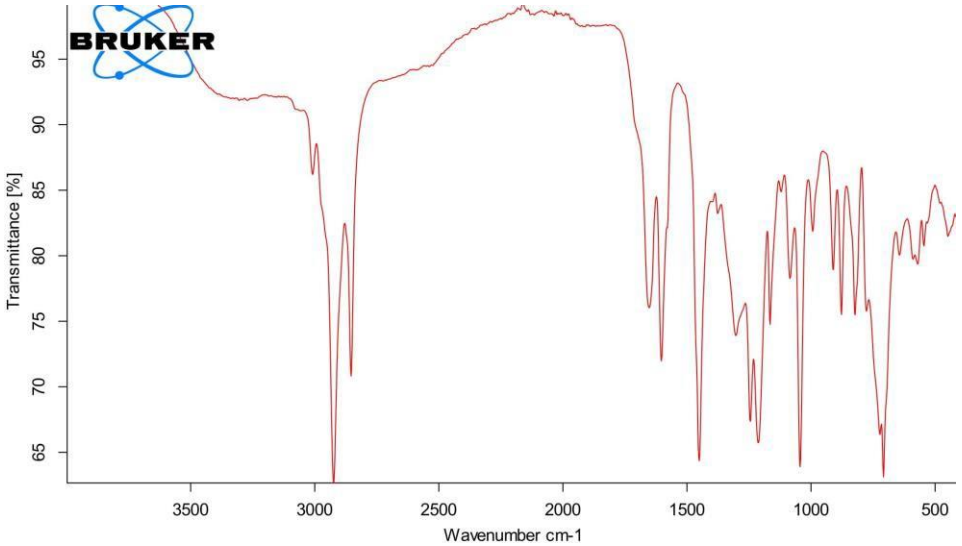

Figure 20: FT-IR chromatogram of CNSL sample gained by ethanol solvent extraction.

In figure 21, an FT-IR chromatogram of CNSL is represented, which used heptane as a solvent.

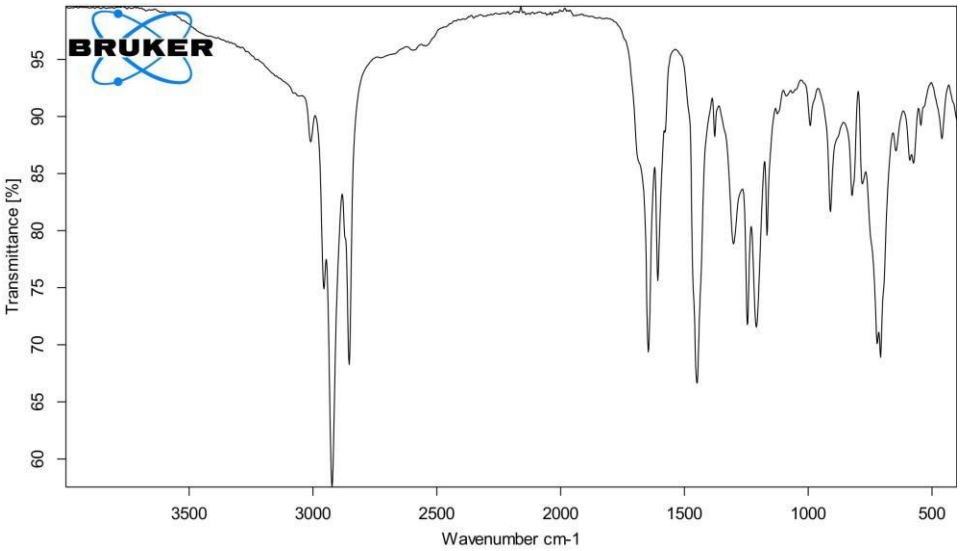

Figure 21: FT-IR chromatogram of CNSL sample obtained by heptane solvent extraction.

The same strong peaks appear at wavenumbers 2900 and 2800 (cm^{-1}), which again indicate the presence of carboxylic acid groups. The chromatogram is similar to the CNSL derived with ethanol. The strong peak around 1100 (cm^{-1}) is the only peak that differs. The peak is most likely indicating an alcohol, which was the solvent used.

Figure 22, shows the FT-IR of the AA, obtained by isolating AA from CNSL. This shows there are small peaks, again at wavenumber 2900 and 2800 (cm^{-1}). These again indicate the presence of carboxylic acid groups. Additionally, an OH-peak at 3400 (cm^{-1}) is visible. These OH-groups can be related to AA as well as to cardol and cardanol since these molecules all contain OH-groups.

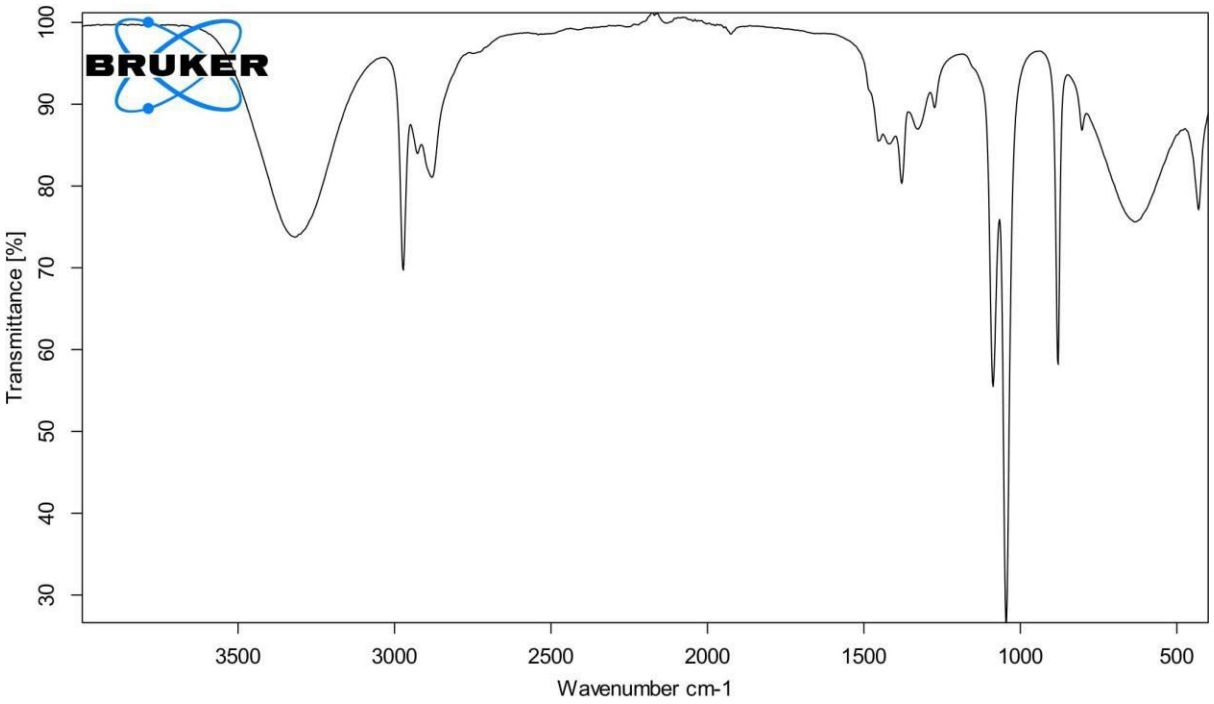

Figure 22: the FT-IR chromatogram of the AA, obtained after using the method for isolation of AA from CNSL.

4.3.3. Gas chromatography (GC)

Using GC-analysis, the amount of ethanol left in the CNSL after evaporation is determined. The concentration is approximately 10 % (v/v) ethanol. After a multi-stage distillation, the amount of ethanol is reduced to approximately 5 %(v/v) ethanol. These concentrations are determined by comparing the area of the ethanol peak in the GC-chromatogram with the calibration curve shown (R^2 of 0,92) in appendix 3 figure 1.

Therefore, the g CNSL/ g CNS derived from the 1-hour extraction at 50°C in the RBR with 7,5 g CNS, particle size 2-4mm, rotating speed 300 rpm and 150 ml ethanol as solvent, is 0,35 instead of 0,39 g CNSL/ g CNS.

4.3.4. Thermogravimetric analysis (TGA)

A TGA-analysis was performed by CoE BBE to determine the percentage of the hemicellulose, cellulose, lignin, polymeric material and CNSL in the CNS before and after extraction. The graphs of the TGA show (%/ºC) on the y-axis and temperature (ºC) on the x-axis. The area represents the percentage of each component in the CNS. The accompanying graph and raw data table can be found in appendix 4. Table 2 shows the composition of the CNS in weight percentages on 100% basis. These values were calculated by adding the area corresponding to the component divided by the total area measured times 100 percent. The mass of the CNSL that is extracted can be calculated using the following formula:

Equation 5:

$$\%\boldsymbol{CNSL\ extracted} = \frac{(\%\boldsymbol{CNSL\ in\ raw\ CNS} - \%\boldsymbol{CNSL\ in\ CNS\ after\ extraction})}{\%\boldsymbol{CNSL\ in\ raw\ CNS}}$$

Table 3: Composition of raw CNS and CNS after extractions.

Sample	CNSL (m%)	Lignin (m%)	Cellulose (m%)	Hemicellulose (m%)	Polymeric material (m%)
Raw CNS	29,3	27,2	9,6	33,9	0,0
60 min, RBR	10,0	26,9	17,5	45,0	0,6
10 min, RBR	23,8	28,1	13,6	33,2	1,3
60 min, stirrer	11,8	28,8	17,4	42,0	0,0

Table 4: Composition of raw CNS and CNS after extractions.

Sample	CNS before extraction (g)	% CNSL in raw CNS	CNS after extraction (g)	% CNSL in the CNS after extraction	CNSL extracted (g)	g CNSL extracted /g CNS	% CNSL extracted
60 min RBR	7,50	29,30	4,80	10,00	1,72	0,23	78,16
60 min stirrer	7,50	29,30	5,00	11,80	1,61	0,21	73,15
10 min RBR	7,50	29,30	5,70	23,80	0,84	0,11	38,27

The highest amount of CNSL extracted was found for RBR-extraction for 60 minutes (0,23 g CNSL extracted/g CNS), while the stirrer showed lower results (0,21 g CNSL extracted/g CNS). This shows that the RBR has an advantage over the stirrer. The extraction for 10 minutes, shows a significantly lower reduction of only 0,11 g CNSL extracted/ g CNS, as presented in figure 23.

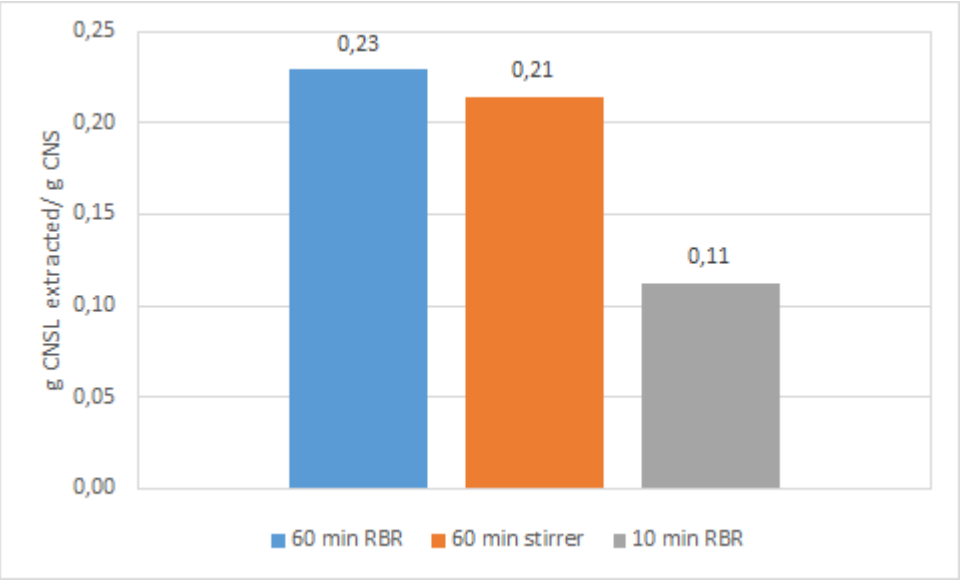

Figure 23: Grams CNSL extracted / grams CNS (according to TGA analysis)

5. Discussion

Using a solvent for the extraction of CNSL, causes an additional step in the process of CNSL-extraction: solvent removal. GC-analysis showed that ethanol content after solvent removal, averaged around 10% (v/v). The heptane content was not determined, therefore the amount of grams CNSL derived from the extraction was not corrected for it. In consequence, the extraction performances (g CNSL/g CNS) are documented higher than they are. Furthermore, by using ethanol as solvent, the composition of the derived CNSL might be different from when a more a-polar solvent is used. This makes the definition of CNSL debatable. This can explain why the CNSL yield is higher than the theoretically maximum yield determined using TGA analysis. TGA showed a total amount of 29,3 m% of CNSL present in raw CNS, while extractions in this project showed a 35 m% recovery of CNSL yield from CNS. However, some papers show [8] CNSL content in CNS to be around 60%. Leaving the validity of the thermogravimetric analysis and the extraction performance in doubt.

Despite the difficulty of determining the definite CNSL amount that is extracted, the relation between the extraction data still reveals information about the optimal conditions for CNSL extraction. The use of particle size distribution (2-4 mm and 6-10 mm) did not have a major influence. This indicates that an equilibrium is reached. The correlation between the amount of CNSL obtained and the solvent/solid ratio is linear also indicating equilibria of CNSL in the solvent and solid. A temperature higher would be in favour of the equilibria for extracting the components to the liquid phase, since higher temperature leads to higher solubility [10]. In figure 7 it is observed, that a lower temperature indeed resulted in a lower CNSL recovery. Because of a large standard deviation of the extraction at 70 °C, it is not evident, that a higher recovery than the extraction at 50 °C was achieved. The rotation speed of the rotating bed showed to be of little influence, indicating that mass transfer from the surface of the CNS to the bulk of the liquid is not limiting the overall mass transfer.

The use of heptane as a solvent appears to be less effective in extracting the CNSL, but as mentioned earlier, the composition of the CNSL derived with heptane might be different. Analysis should determine the AA amount present in the CNSL that has been extracted. In this way, the two solvents can be compared. To determine to amount of heptane left in the solvent a GC analyses on the sample and a calibration needs to be executed.

The correlations of CNSL yield in the RBR with different extraction times, can be seen as a rough indication. The extractions have only been performed in twofold (with some exceptions). Furthermore, no extractions of 120 minutes, using the regular stirrer, were performed. For the rotating bed extractions, no extractions were performed using extraction times of 2, 4 and 8 minutes. This might have an influence on the reliability of the correlations. Especially since the 50-minute extraction, performed with regular stirring, differs from the trend line. The mass loss of the extraction showed significant higher loss for extractions where the solid and liquid had to be separated using a filter. This loss is mainly ethanol that evaporates during the filtration. The loss of solvent does not influence the yield of CNSL that is determined in the end.

GC-analysis showed that the ethanol content in the CNSL averaged around 10% (v/v). Considering the density of the natural CNSL is 1,009 g/cm^3, presents the m% around 8. A fractional distillation was done to reduce the ethanol content. This resulted in a final ethanol concentration of roughly 5 %(v/v), so now the m% is around 4. In order to determine the exact amount of ethanol in the CNSL, a more detailed analysis is needed. An internal standard should be added to the samples when using the GC. The calibration line will be more accurate, and the precise ethanol content can be determined. Nevertheless, for this experiment, only a rough estimate was needed to determine if a multistage distillation of the CNSL was required.

The composition of the cashew nut shell liquid (CNSL) was analyzed using HPLC. The chromatograms showed peaks at specific retention times, which can be related to known components. Comparing the

chromatogram of the CNSL sample using solvent extraction, to the chromatogram of AA triene standard, technical CNSL, cardanol and the saturated AA standard. The chromatograph of the CNSL extracted by solvent have peaks at the retention time of 3 min. is cardol; 6,5 is AA triene ; 7,3 is cardanol; 9,7 is AA diene and 16,2 is AA monoene.

The concentration of the AA triene of the sample nº16 is 83,1 mg AA triene /ml of CNSL. Considering a density of 1,009 g/cm^3, the m% is 8,2. This weight percentage is lower than expected, because according to literature [4][15], the percentage should be around 28 m%.

6. Conclusion

The goal of this project was to design an extraction process, which could extract CNSL from cashew nut shells (CNS) with a yield of ≥90 m%. Using a rotating bed reactor, CNSL has been extracted with a yield of 78,16 m%. The highest extraction performance of CNSL was found with an extraction time of 1 hour, at 70 °C, using a stirring speed of 300 rpm, 150 ml ethanol, 7,5 grams of CNS and a particle size distribution of 2-4 mm. The yield reached 78,16 m%, according to TGA analysis. Therefore, the goal of >90 m% recovery was not achieved.

When the reactor temperature is decreased, the CNSL extraction yield decreases. Increasing the temperature results in a higher yield. The particle distribution size does not have an influence on the extraction performance. Therefore, less pre-treatment is required, the CNS 6-10 mm can be used instead of the 4-6 mm CNS. Increasing the amount of solvent, and therefore, decreasing the solid-liquid ratio, increases the CNSL extraction yield. Using a different solvent, heptane, resulted in a decrease of ~ 8% v/v. Most ethanol (~ 90 m/m%) can be recovered with the purification of the CNSL using multistage distillation.

HPLC-analyses shows the presence of anacardic acids in the extract. With the use of analytical standards and a calibration line, the concentration of AA triene in the CNSL was determined at 83 mg/ml.

7. Recommendations

It is recommended to continue investigating the extraction performance of CNSL extraction out of CNS. Using a rotating bed under mild conditions, results in a yield <90%. Therefore, it is suggested that the before mentioned extraction techniques (ASE, SCF) are examined. Furthermore, when using solvent extraction, the optimal solvent/solid ratio has to be examined further, since in this project there have only been decreases in ratio, when compared to the base case extraction, and no increases.

The recovery of ethanol after extractions is not efficient in this process. When a more efficient method for ethanol recovery can be implemented, a higher extraction performance can be reached. When costs of this extraction process are evaluated, solvent is the most expensive product. Therefore, in order to make the extraction more economically attractive and feasible on a bigger scale, more ethanol has to be recovered.

In order to monitor the extraction performance, a more comprehensive analytical method for component determination is recommended. During this project, only the saturated and triene AA were present as analytical standard, while CNSL only contained the latter. This way, yield of the other AA forms were unable to be determined.

Literature

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Appendixes

Appendix 1 Mass balance extractions

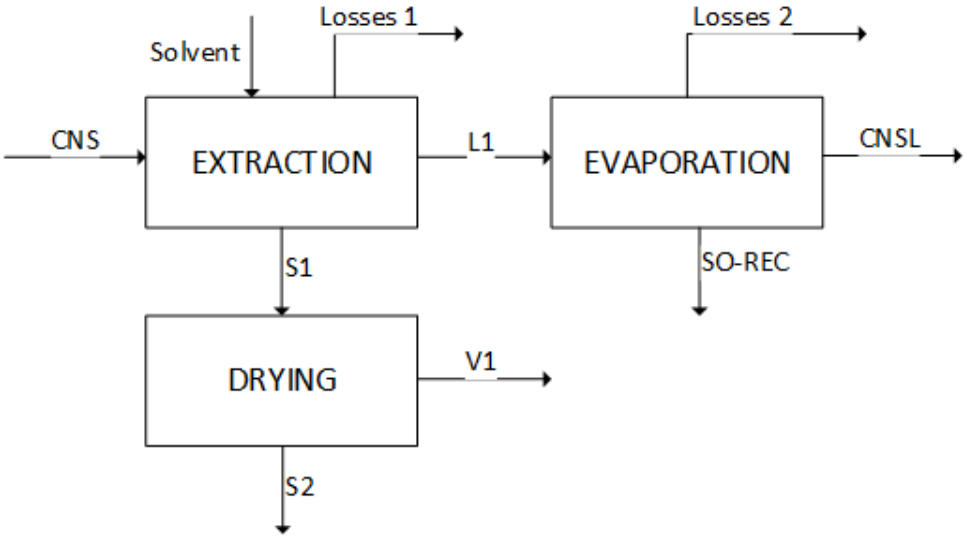


Figure 1: overview of the process with stream names.

Table 1 shows all mass streams for the extraction step documented according figure 1. Table 2 shows all the mass streams for the evaporation step documented according figure 2. Table 3 shows all mass streams for the drying step documented according to figure 3.

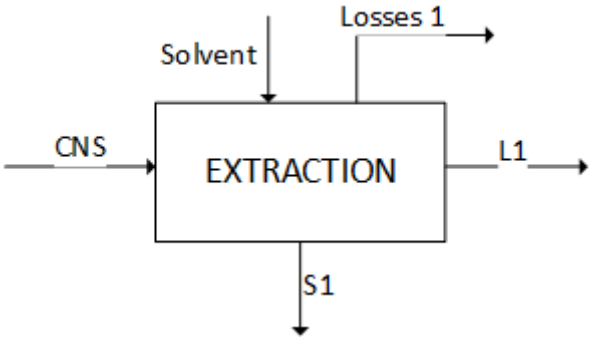


Figure 2: Extraction step overview with stream names

Table 1: Total Mass balance of the extraction step. According mass stream names from figure 2.

parameter	condition	duplo	CNS (g)	Solvent (g)	L1 (g)	S1 (g)	Losses 1 (g)
none	standard	1	7,5	115,4	108,3	10	4,6
none	standard	2	7,5	115,7	108,1	10	5,1
Particle size	6-10 mm	1	7,5	115,5	110,4	9,5	3,1
Particle size	6-10 mm	2	7,5	115,5	111,2	9,2	2,6
Solvent	Heptane	1	7,5	99,4	95,5	7,9	3,5
Solvent	Heptane	2	7,5	99,4	91,9	8,7	6,3
stirring speed	0 rpm	1	7,5	115,2	108,1	5,2	9,4
stirring speed	0 rpm	2	7,5	115,1	107,6	5,1	9,9
stirring speed	1050 rpm	1	7,5	115,8	110	8,2	5,1
stirring speed	1050 rpm	2	7,5	115,7	108,5	9,8	4,9
set-up	regular stirrer	1	7,5	115,4	98	8	16,9
set-up	regular stirrer	2	7,5	115,4	97,9	7,5	17,5
solvent/solid ratio	6,67 ml/g	1	7,5	39	24,2	6,1	16,2
solvent/solid ratio	6,67 ml/g	2	7,5	38,8	24,9	6,3	15,1
solvent/solid ratio	13,33 ml/g	1	7,5	78,3	68,5	5,2	12,1
solvent/solid ratio	13,33 ml/g	2	7,5	78,2	67,8	5,3	12,6
temperature	20 °C	1	7,5	115,5	110,4	9,5	3,1
temperature	20 °C	2	7,5	115,6	110,3	9,6	3,2
temperature	70 °C	1	7,5	115,7	110	8,6	4,6
temperature	70 °C	2	7,5	115,4	111,5	9,5	1,9
time	10 min	1	7,5	115,7	111,6	9	2,6
time	10 min	2	7,5	115,6	110,9	9,5	2,7
time	20 min	1	7,5	115,8	105,4	9,3	8,6
time	20 min	2	7,5	115,6	107,5	9,6	6
time	30 min	1	7,5	115,5	108,8	7,6	6,6
time	30 min	2	7,5	115,7	108,9	7,9	6,4
time	40 min	1	7,5	115,7	108,4	10,2	4,6
time	40 min	2	7,5	115,2	109,9	10,5	2,3
time	50 min	1	7,5	115,4	104		18,9
time	50 min	2	7,5	115,5	100	9,6	13,4
time	120 min	1	7,5	115,7			123,2
time	120 min	2	7,5	115,5			123
time and set-up	2 min	1	7,5	115,3	104	5,1	13,7
time and set-up	2 min	2	7,5	115,2	104,5	5	13,2
time and set-up	4 min	1	7,5	115,4	105,6	4,8	12,5

time and set-up	4 min	2	7,5	115,6	105,4	5,1	12,6
time and set-up	8 min	1	7,5	115,5	105,3	5,1	12,6
time and set-up	8 min	2	7,5	115,3	104,6	4,9	13,3
time and set-up	10 min	1	7,5	115,3	110	5,9	6,9
time and set-up	10 min	2	7,5	115,1	107,8	5,1	9,7
time and set-up	20 min	1	7,5	115,2	108,4	5,1	9,2
time and set-up	20 min	2	7,5	115,2	107,5	4,9	10,3
time and set-up	30 min	1	7,5	115,3	106,8	5	11
time and set-up	30 min	2	7,5	115,5	107,1	5	10,9
time and set-up	40 min	1	7,5	115,5	107,6	5,1	10,3
time and set-up	40 min	2	7,5	115,7	107,41	4,9	10,89
time and set-up	50 min	1	7,5	115,6	101,2	9,5	12,4
time and set-up	50 min	2	7,5	115,3	102,6	11,6	8,6

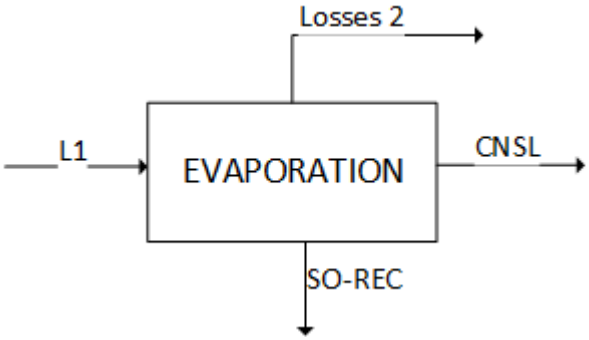


Figure 3: Overview of the evaporation step

Table 2: Total Mass balance of the evaporation step. According to mass stream names from figure 3.

parameter	condition	duplo	L1 (g)	CNSL (g)	SO-REC (g)	Losses 2 (g)
none	standard	1	108,3	2,9	102	3,4
none	standard	2	108,1	3	105	0,1
Particle size	6-10 mm	1	110,4	3	104	3,4
Particle size	6-10 mm	2	111,2	3	100	8,2
Solvent	Heptane	1	95,5	2,8	89	3,7
Solvent	Heptane	2	91,9	2,6	85	4,3
stirring speed	0 rpm	1	108,1	2,6	104	1,5
stirring speed	0 rpm	2	107,6	2,6	104	1
stirring speed	1050 rpm	1	110	3,5	102	4,5
stirring speed	1050 rpm	2	108,5	2,6	107	-1,1
set-up	regular stirrer	1	98	3,1	90	4,9
set-up	regular stirrer	2	97,9	2,8	70	25,1
solvent/solid ratio	6,67 ml/g	1	24,2	2,2	20	2
solvent/solid ratio	6,67 ml/g	2	24,9	2	20	2,9
solvent/solid ratio	13,33 ml/g	1	68,5	2,5	65	1
solvent/solid ratio	13,33 ml/g	2	67,8	2,4	64	1,4
temperature	20 °C	1	110,4	2,4	70	38
temperature	20 °C	2	110,3	2,5	80	27,8
temperature	70 °C	1	110	3,2	103	3,8
temperature	70 °C	2	111,5	2,8	105	3,7
time	10 min	1	111,6	2,2	107	2,4
time	10 min	2	110,9	2	107	1,9
time	20 min	1	105,4	2,2	107	-3,8

time	20 min	2	107,5	2,5	104	1
time	30 min	1	108,8	2,6	104	2,2
time	30 min	2	108,9	2,5	105	1,4
time	40 min	1	108,4	2,7	104	1,7
time	40 min	2	109,9	2,8	105	2,1
time	50 min	1	104	2,6	97	4,4
time	50 min	2	100	2,8	98	-0,8
time	120 min	1		3,1		-3,1
time	120 min	2		3	95	-98
time and set-up	2 min	1	104	2,1	103	-1,1
time and set-up	2 min	2	104,5	2,2	102	0,3
time and set-up	4 min	1	105,6	2,3	103	0,3
time and set-up	4 min	2	105,4	2,3	104	-0,9
time and set-up	8 min	1	105,3	2,3	101	2
time and set-up	8 min	2	104,6	2,4	103	-0,8
time and set-up	10 min	1	110	2,3	104	3,7
time and set-up	10 min	2	107,8	2,4	104	1,4
time and set-up	20 min	1	108,4	2,5	104	1,9
time and set-up	20 min	2	107,5	2,4	105	0,1
time and set-up	30 min	1	106,8	2,7	103	1,1
time and set-up	30 min	2	107,1	2,5	104	0,6
time and set-up	40 min	1	107,6	2,7	104	0,9
time and set-up	40 min	2	107,41	2,6	105	-0,19
time and set-up	50 min	1	101,2	2,7	99	-0,5
time and set-up	50 min	2	102,6	2,6	103	-3

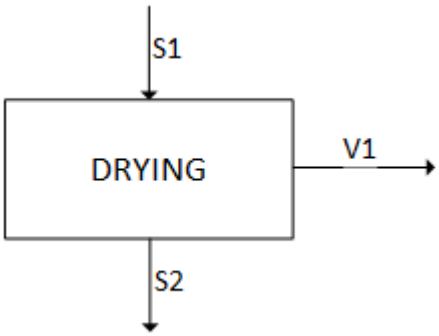
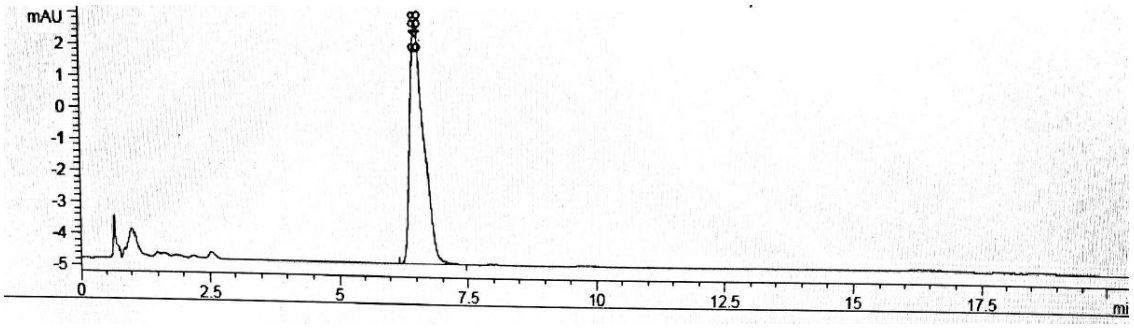


Figure 4: Overview of the drying step

Table 3: Total Mass balance of the drying step. According mass stream names from figure 4.

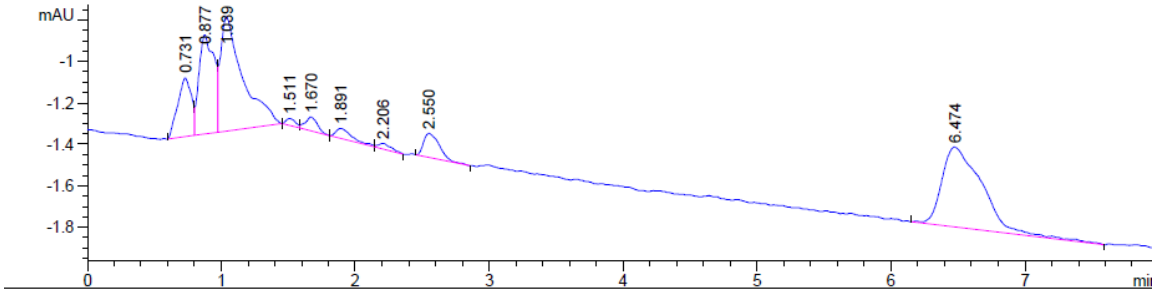
parameter	condition	duplo	S1 (g)	S2 (g)	V1 (g)
none	standard	1	10	4,7	5,3
none	standard	2	10	4,8	5,2
set-up	regular stirrer	1	8	5,1	2,9
set-up	regular stirrer	2	7,5	5	2,5
time	10 min	1	9	5,7	3,3
time	10 min	2	9,5	5,6	3,9

Appendix 2: HPLC results



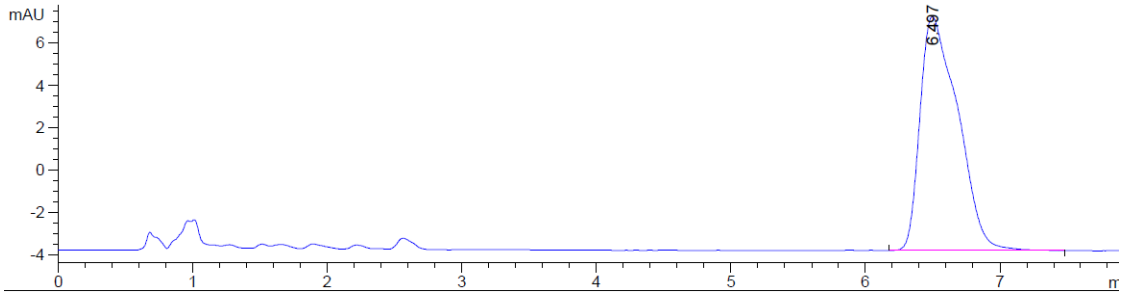
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.488	BB	0.2669	139.26837	7.52432	100.0000
Totals :				139.26837	7.52432	

Figure 1: 0,1 mg/ml AA triene standard on HPLC



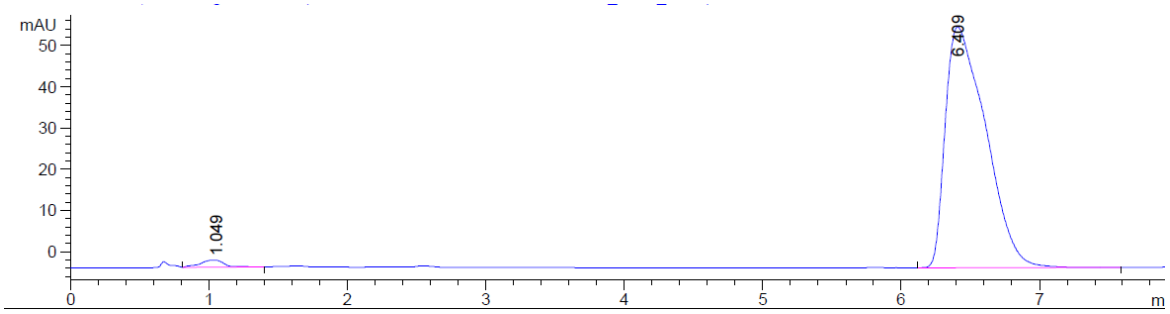
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.731	BV	0.1028	2.00332	2.83570e-1	8.9114
2	0.877	VV	0.1119	3.75710	4.79060e-1	16.7127
3	1.039	VB	0.1564	6.43915	5.52463e-1	28.6432
4	1.511	BV	0.0831	1.71656e-1	3.35011e-2	0.7636
5	1.670	VB	0.0963	4.17807e-1	6.67581e-2	1.8585
6	1.891	BV	0.1156	4.02732e-1	4.86275e-2	1.7915
7	2.206	VB	0.0931	1.79574e-1	2.66880e-2	0.7988
8	2.550	BB	0.1338	9.48125e-1	1.15301e-1	4.2175
9	6.474	BBA	0.2919	8.16106	3.85858e-1	36.3028

Figure 2: 0,05 mg/ml AA triene standard on HPLC



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.497	BB	0.2762	215.84006	11.03712	100.0000

Figure 3: 0,2 mg/ml AA triene standard on HPLC



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.049	VB	0.1694	21.82816	1.72626	1.8083
2	6.409	BB	0.2878	1185.27747	58.39617	98.1917

Figure 4: 0,5 mg/ml AA triene standard on HPLC

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.676	BV	0.0500	20.71926	6.29588	4.3740
2	0.724	VV	0.0584	19.21757	4.64714	4.0569
3	3.001	BB	0.1541	41.39275	4.05284	8.7382
4	6.472	BB	0.2782	178.43263	9.22096	37.6682
5	9.695	BB	0.3693	58.30787	2.28346	12.3091
6	16.182	BB	0.6218	155.62613	3.55245	32.8536

Figure 5: retention time CNSL sample 30 minutes, 150 ml ethanol, 7,5 gram CNS, RBR

Appendix 3: GC analysis calibration curve

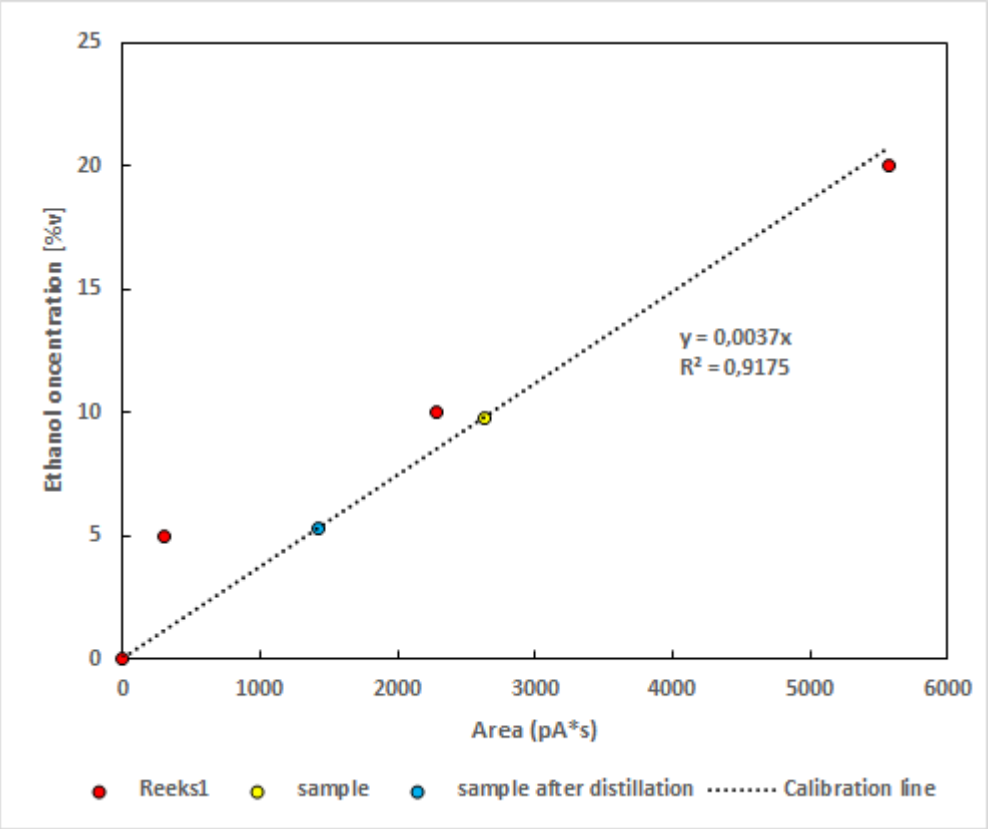


Figure 1: Calibration curve for determination of ethanol (v/v%) left in sample after evaporation.

Table 1: Calibration line and sample nº 4 before and after the distillation

Calibration line	
Concentration of ethanol (%v/v)	Area (pA*s)
0	0
5	296,33972
10	2281,60487
20	5578,55762
50	3,32E+04
sample nº 4 before distillation	
Area (pA*s)	2632,84033
Concentration of ethanol (%v/v)	9,741509221
sample nº 4 after distillation	
Area (pA*s)	1427,2
Concentration of ethanol (%v/v)	5,28064

Appendix 4: TGA analysis results

Model	Gaussian									
Plot	*CNSL*			*Hemicellulose*			*Cellulose*		*Polymeric Materi*	*Lignin*
y0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
xc	177,5 ± 0	195 ± 0	215,20861 ± 0	227,3213 ± 0	240,51847 ± 0	272,56125 ± 0	307,51881 ± 0	327,13244 ± 0	431,67056 ± 0	386,98127 ± 0
A	1,0341 ± 0,0088	0,91924 ± 0,0062	3,212 ± 0,0042	1,71373 ± 0,0024	5,04564 ± 0,0040	16,46355 ± 0,004	7,7861 ± 0,0034	1,25334 ± 0,0033	0,25925 ± 0,0073	13,86953 ± 0,020
w	30 ± 0	20 ± 0	20 ± 0	9,32611 ± 0	25 ± 0	38,5 ± 0	22,10717 ± 0	14,03413 ± 0	30 ± 0	185 ± 0
Reduced Chi-Sq	1,7495E-5									
R-Square (COD)	0,99918									

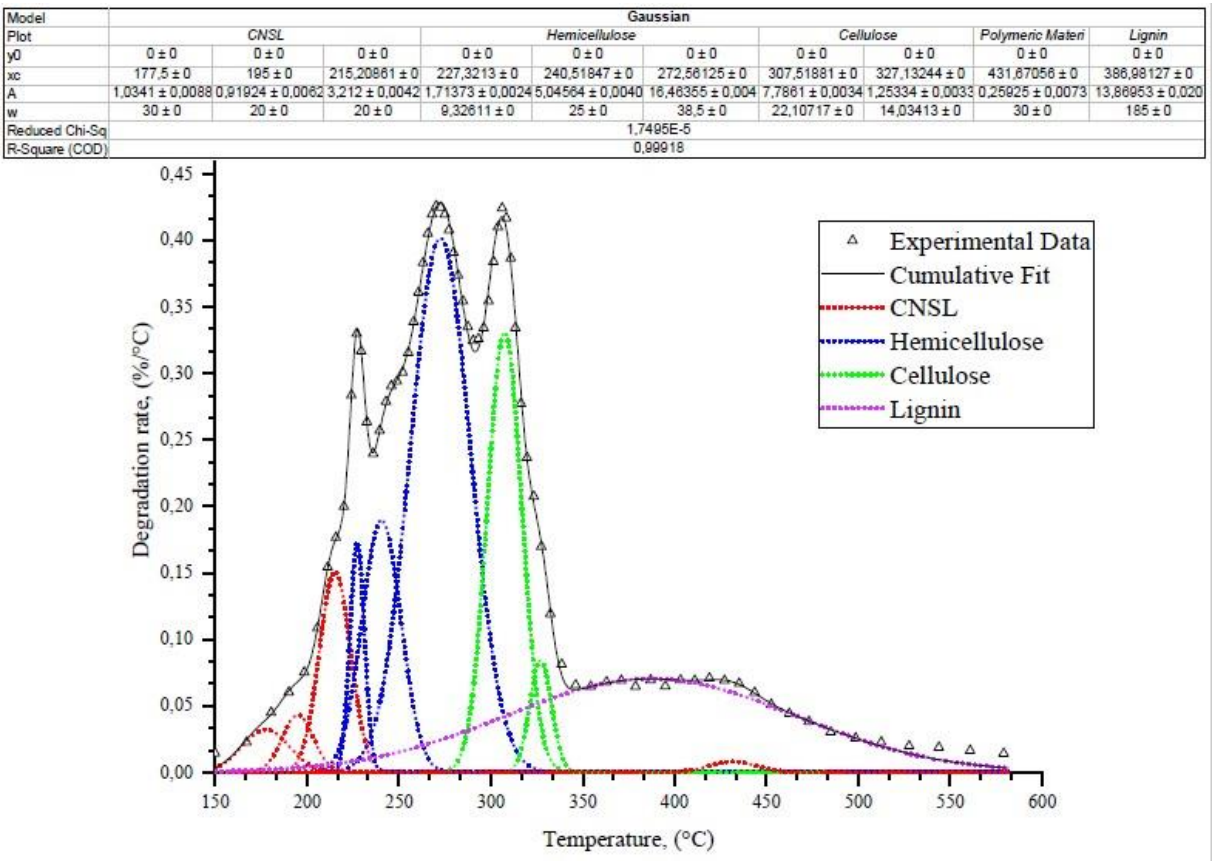


Figure 1 TGA analyis of 1 hour RBR extraction.

The percentages were calculated by adding all areas of a component and dividing it by the total area of all peaks found.

Model	Gaussian									
Equation	y = y0 + A/(w*sqrt(pi/(4*ln(2)))) * exp(-4*ln(2)*(x-xc)^2/w^2)									
Plot	CNSL			Hemicellulose			Cellulose		Polymeric Materi	Lignin
y0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
xc	171 ± 0	190,59344 ± 0	211,98795 ± 0	225,14255 ± 0	242 ± 0	277 ± 0	303,94577 ± 0	322,8979 ± 0	422,57104 ± 0	381,35483 ± 0,366
A	2,57541 ± 0,00	9,69259 ± 0,007	3,27703 ± 0,009	1,59756 ± 0,005	6,24134 ± 0,013	13,82262 ± 0,020	7,83773 ± 0,006	1,00506 ± 0,006	0,84206 ± 0,013	18,31224 ± 0,0671
w	30 ± 0	24,56992 ± 0	25 ± 0	13,60968 ± 0	29,87328 ± 0	38 ± 0	15,69773 ± 0	11,61526 ± 0	31,6224 ± 0	190 ± 0,85713
Reduced Chi-S	4,40847E-5									
R-Square (COD	0,99822									
Adj. R-Square	0,99822									

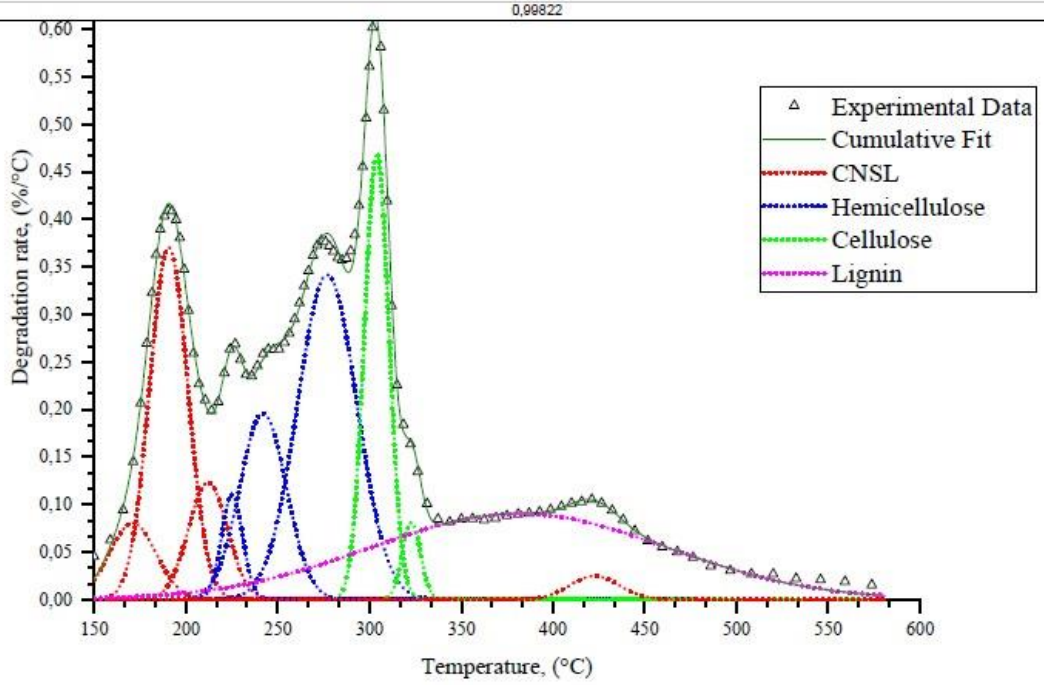


Figure 2: TGA analysis of 10 min extraction, RBR extraction

The percentages were calculated by adding all areas of a component and dividing it by the total area of all peaks found.